ARCHIVES OF DISEASE IN CHILDHOOD

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STUDIES IN THE ANÆMIAS OF INFANCY AND EARLY CHILDHOOD

(From the Children's Hospital and the Department of Diseases of Children of the University, Birmingham).

Part I.—Introduction

BY

LEONARD G. PARSONS, M.D., F.R.C.P.

The last decade has witnessed a great revival of interest in diseases of the blood due primarily to the epoch-making discoveries in pernicious anæmia by Minot and Murphy, which in their turn developed out of the work of Whipple and Robscheit-Robins. This renaissance of hæmatology has included the anæmias of childhood, and for the new and increasing knowledge that we possess of these disorders we are chiefly indebted on the clinical side to our country woman Helen Mackay, and on the experimental side to Hart, Steenbock, Elvehjem and Waddell of the University of Wisconsin, U.S.A. During the past three years at the Children's Hospital, Birmingham, my colleagues and I have been engaged in an attempt to apply the results obtained by other workers and ourselves in the treatment of nutritional anæmia in rats, to the elucidation of some of the problems of nutritional and other anæmias in the infant and young child. Our experimental results with the description, classification and therapeutics of the various clinical forms of anæmia we have encountered, are recorded in the present series, under the following headings:—*

Part I. Introduction.
Part II The effect of yeast on nutritional anæmia in the rat.
Part III. Anhæmatopoietic anæmias (deficiency diseases of the erythron): nutritional anæmia and the anæmias of prematurity, scurvy and cœliac disease.
Part IV. Hæmolytic (erythronoclastic) anæmia of the neonatal period.
Part V. Hæmolytic (erythronoclastic) anæmia of later infancy and childhood.
Part VI. Anæmia in mother and child.
Part VII. Monocytosis in myelosis.
Part VIII. Leukæmia.
Part IX. Reticulo-endotheliosis in severe anæmia.
Part X. The hæmatocrit in clinical medicine.

* Parts IV—X will appear in the next and ensuing numbers.

J. S. Haldane[1] has recently written ' the blood in the living body is a living structure actively maintained,' and this conception of the blood as not merely a vehicle for the conveyance of material to and from the body cells, but as a tissue or organ, consisting of not only the circulating cells but also of their predecessors, has led to a better understanding of many of the problems presented by the anæmias. This point of view has been particularly stressed by Boycott[2] and Witts[3], and the former has suggested the word ' erythron ' to connote ' the tissue which is made up of the circulating red cells and the cells in the bone marrow (and sometimes elsewhere) from which they arise.' The cells, therefore, which comprise the erythron are the reticulo-endothelium of the marrow and extra-medullary hæmopoietic centres, megaloblasts, normoblasts, reticulocytes and erythrocytes.

The erythron in early childoood.

Hæmatopoietic centres.—The erythron in infancy is anatomatically even more widely spread than in the adult, because although the change over from the embryonic depôts of blood formation occurs during the later months of gestation, this change is gradual and blood formation continues to take place in the shafts of the long bones, the flat bones, the liver, spleen and lymphatic tissues throughout the early months of post-natal life. There is thus little, if any, reserve bone-marrow space in the infant, and therefore in time of stress the extra-medullary centres in the liver, spleen, pancreas, etc., may be recalled into activity. There may even be actual absorption of bone to increase the marrow space, and this to an extent which may be demonstrable in a radiograph.

With the onset of adolescence the red bone marrow begins to recede from the long bones and to be replaced by fat. According to Piney[4], whose observations we can confirm, the red bone marrow in the shafts of the long bones is pink in colour from birth until the age of three or four years; at seven years it becomes less pink and the surface appears greasy, but no macroscopic fat is visible until the age of twelve or fourteen years. These fatty changes occur more rapidly in the long bones of the lower than in those of the upper extremity. To ensure the normal development of the red cell certain factors have to be supplied to the hæmatopoietic centres. For the maturation of the normoblast to the erythrocyte Witts points out that iron, copper, Vitamin C and thyroxin are all necessary. The recent work of Strauss and Castle[5] shows that the maturation from megaloblast to normoblast can only occur in the presence of a ' hæmatinic factor,' formed by the interaction of an intrinsic factor present in normal gastric juice, and an extrinsic factor which they believe to be B_2 or a substance closely allied to it.

Red blood cells.—On account of the anoxæmia of intra-uterine life the infant at birth has a blood of high concentration, the erythrocytes numbering about 7 millions per c.mm., and the hæmoglobin as estimated by Haldane's method being often 120 per cent., or even higher. During the first week of extra-uterine life the number of red cells falls to between

5 and 6 millions, perhaps nearer to 6 than 5 millions, and during the period of infancy remains at that figure; further, this fall in red cells occurs whether the child is born by Cæsarean section or naturally. In the embryo the blood contains many nucleated red cells, a state of affairs which may be present at birth in the condition known as erythroblastosis of the new-born. Normally, however, the numbers of normoblasts diminish in the later stages of pregnancy, and although present at birth they soon

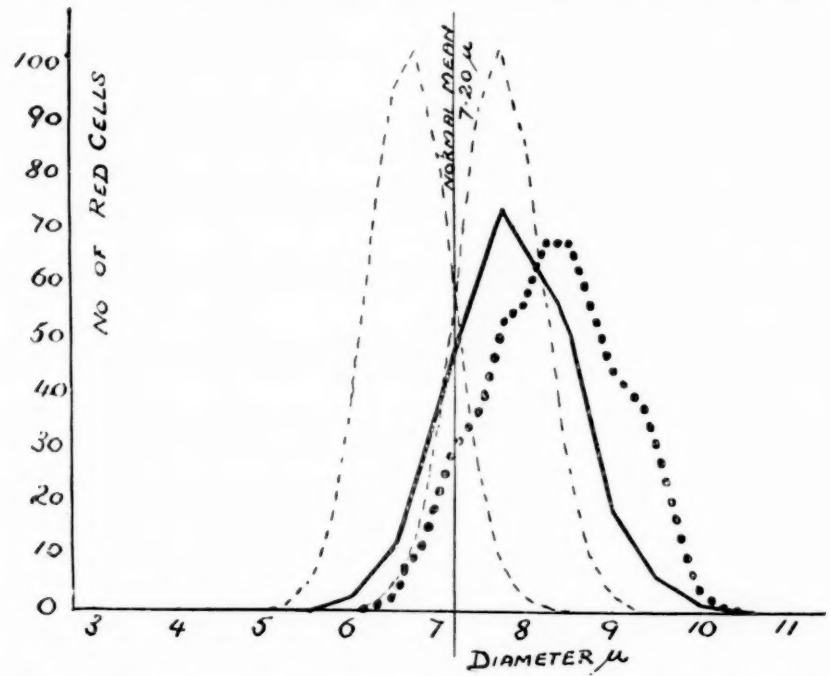


Fig. 1.—Price-Jones curves constructed from data given by van Creveld[6].

- - - - Ideal curves for the smallest (6·686μ) and largest (7·718μ), mean diameters within normal limits for adults and children.

. . . . Curve for full term infants at the 4th week of life. Mean diameter 8·266μ.

—— Curve for premature infants at the 4th week of life. Mean diameter 7·912μ.

disappear but may return in response to abnormal stimuli. At birth the red cells show variations in size and shape, and van Creveld[6], by means of Price-Jones curves, has investigated the changes which occur in them during the first few weeks of life, both in full-term and premature infants. During the first week of life the curves of premature and full-term children are similar and show a megalocytic type of curve with a greater degree of anisocytosis than occurs in older children or adults. In the second week of life the curves show little difference except that the mean diameter of the red cells increases a little. During the third and following weeks the curves of premature and full-term children separate, the mean diameter of the red cells decreasing in the premature children and anisocytosis being more marked than in full-term children (Fig. 1).

In full-term children the mean diameter of the red cells decreases in the fifth week and remains about constant from the sixth week onwards (Fig. 2), anisocytosis becomes definitely less, and thereafter the curve approaches more and more the adult type. The mean diameter of the red cells in premature children reaches its smallest figure at the age of eight weeks and after that time the anisocytosis slowly becomes less after the tenth week, a slow increase in the mean diameter occurs, and probably about the eighteenth to twentieth week the curves of premature and full-term children are identical.

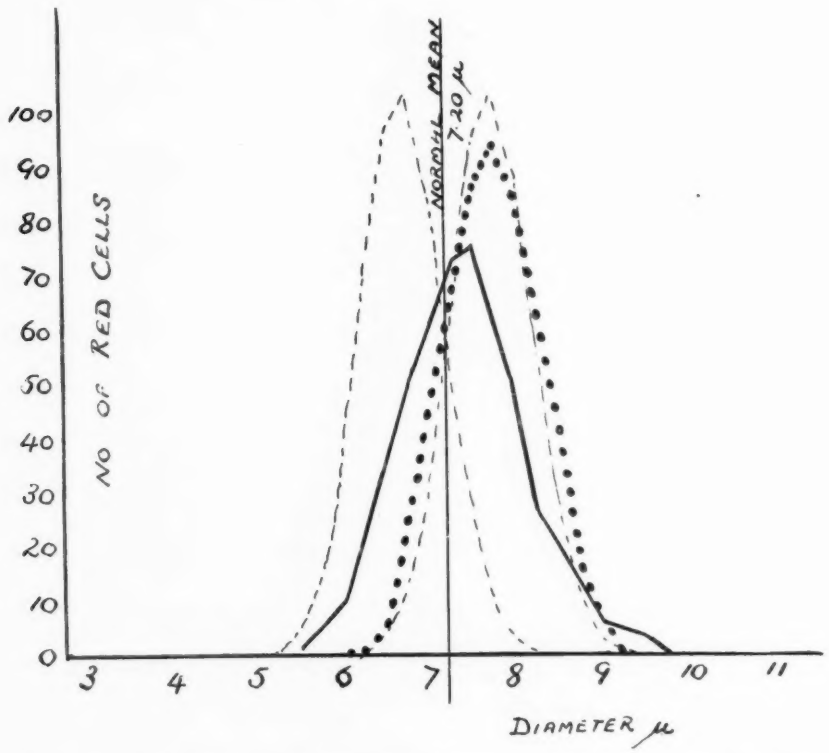


FIG. 2.—Price-Jones curves constructed from data given by van Creveld[6].

- - - - Ideal curves for the smallest (6·686μ) and largest (7·718μ), mean diameters within normal limits for adults and children.

. . . . Curve for full term infants at the 6th week of life. Mean diameter 7·7208μ.

—— Curve for premature infants at the 6th week of life. Mean diameter 7·547μ.

The life of a red blood cell, according to Rous, is about three weeks, when, worn out by the buffeting it has received in the peripheral circulation, it breaks up. The débris of the red cells is removed by the reticulo-endothelial cells of the spleen, liver, and bone marrow, which split the hæmoglobin into bilirubin and an iron-containing fragment. The iron-containing fragment is stored as hæmosiderin in the liver and spleen,

and is used for the formation of fresh hæmoglobin; the bilirubin is excreted by the liver. With excessive hæmolysis the amount of bilirubin may be too much for the liver to excrete and jaundice occurs (' retention jaundice ' of Rich[7]); jaundice produced in this way gives an indirect van den Bergh reaction in the blood serum. The bilirubin excreted into the intestine is in great part converted by the action of bacteria into urobilinogen, but a small part is re-absorbed. The greater part of the urobilinogen is excreted in the fæces, but some of it is also re-absorbed, taken to the liver and reconverted into bilirubin. If the amount of urobilinogen is excessive, as occurs when a large amount of bilirubin due to hæmolysis is excreted by the liver, it passes into the general circulation and is excreted in the urine in which it may be oxidized to urobilin. In this way the presence of an excess of urobilin or of urobilinogen in the urine is indicative of hæmolysis.

The reduction in the number of erythrocytes at birth is brought about by a process of hæmolysis. The degree of hæmolysis and of the resultant bilirubinæmia varies considerably; usually it is either insufficient to produce obvious signs or is enough to cause a jaundice of varying degree, the physiological jaundice of the new born (icterus neonatorum). According to Goldbloom and Gotlieb[8] the fragility of the red cells directly after birth is greater than normal, and their resistance only increases after the appearance of icterus. These authors have also been able to demonstrate experimentally the truth of the statement that icterus neonatorum is due to the change from the anoxæmia of intrauterine life to conditions in which the oxygen supply is much greater. Their experiments consisted in keeping guinea pigs for two or three weeks in an atmosphere at less than normal pressure, and then transferring them to one of normal pressure, thus subjecting them to the same change, as far as oxygen tension was concerned, as occurs in the passage of the fœtus from the pre-natal to the post-natal state. As a result of this change there was a fall in the number of red cells and bilirubinæmia occurred.

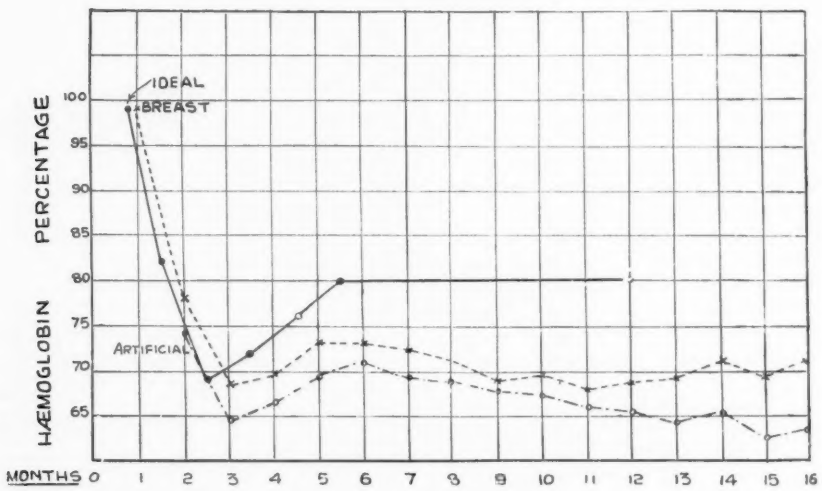
Sometimes in the new-born child hæmolysis is severe and unchecked, and a profound anæmia of the hæmolytic type develops within a few days of birth. This form of hæmolysis occurs in familial icterus gravis. As a result the bone marrow is stimulated and the extra-medullary centres of the erythron recalled into activity and a form of erythroblastosis of the new born is produced. Apart from this syndrome there are, however, milder degrees of hæmolytic anæmia in early infancy for which this process is responsible, and these, according to van Creveld and Heybroek[9], are met more frequently in premature than in full-term infants.

Hæmoglobin.—The hæmoglobin also falls after birth although this fall continues over a longer period than the drop in the red cells. The fall in the hæmoglobin has been the subject of extensive study by Helen Mackay who has constructed three curves representing: (a) the ideal

hæmoglobin curve throughout infancy which is produced when the child takes an adequate amount of iron: this is the highest of the three curves; (b) an intermediate curve representing the hæmoglobin in breast-fed infants; and (c) the lowest curve representing the hæmoglobin in the artificially fed. These curves are shown in Graph I. By regarding the highest curve as the 'ideal' normal and the others as the 'normal' normal for breast and artificially fed infants respectively, it is possible to classify infants as short of circulating hæmoglobin or not according to their age and diet. The question whether or not the 'ideal' normal or the 'normal' normal for breast or artificially-fed children is the 'physiological' normal has been argued, but it would appear that a hæmoglobin percentage which is attained when a healthy child of two years is on a mixed diet cannot be abnormal, and such a figure can only be obtained during the lactation period if the 'ideal' normal curve is achieved.

GRAPH I.

HÆMOGLOBIN CURVES IN INFANCY (MACKAY).



.———. Ideal curve: infants with adequate iron intake.
×- - -× Curve in breast-fed infants.
o—o—o Curve in artificially-fed infants.

While it is true that the theory of early hæmolysis has usually been accepted as the explanation of the diminution of the red cells, the fall in hæmoglobin is by some authorities said to be due rather to diminution in the rate of formation than to an increased destruction of the blood. According to Josephs[10] this is shown by the fact that administration of iron during the early period of falling hæmoglobin appears to have no influence on reticulocytes nor on the fall in hæmoglobin and red cells. He regards this lessening of the rate of blood formation as physiological and leading to the adjustment of the rates of blood formation and destruction at a lower level than obtains at birth, the fall reaching a point below that at which equilibrium is eventually adjusted.

Diseases of the erythron in infancy and early childhood.

Like other tissues the erythron is prone to disease. Morphologically it may show hyperplasia, hypoplasia, or aplasia of the bone marrow. Witts postulates two degrees of hyperplasia: (a) the normoblastic reaction in which the number of normoblasts in the circulating blood is greatly increased, and in the adolescent child the red marrow spreads into the shafts of the long bones; (b) the megaloblastic reaction, a more profound change in which not only is the red marrow increased as in the normoblastic reaction, but the level of cell differentiation is shifted back to an earlier stage in which megaloblasts proliferate actively and even appear in the peripheral blood. In hyperplasia of the marrow nucleated red cells may easily be washed into the blood stream and appear in the circulating blood, but an increased turn-over of red cells does not necessarily occur; indeed, there is often a diminished output of cells because they fail to mature. The presence of nucleated red cells in the blood stream is therefore only evidence of hyperplasia of the marrow, not of increased blood formation, but if reticulocytes are also present then increased blood formation is taking place. The routine examination for reticulocytosis and the estimation of its degree is of the greatest importance in the study of anæmia, and any therapeutic measure which does not result in a definite reticulocytosis within four to six days from the time of its application must be written down as inadequate.

A clinical study of the anæmias of infancy shows that the majority may be classified into two main groups: (1) those in which there is a deficiency in one or more of the elements necessary for the synthesis of hæmoglobin, or for the production of the erythrocyte, or for both of these (the deficiency or anhæmatopoietic anæmias); and (2) those in which there is increased destruction of the erythronic elements (the hæmolytic or erythronoclastic anæmias).

It is possible that a deficiency anæmia may arise even when the diet supplies all the elements necessary for the production of red cells with their normal content of hæmoglobin since absorption from the alimentary tract may be defective. Again, even with adequate absorption the body, for some reason, as pointed out by Josephs, may fail to carry out the synthesis of hæmoglobin. The anæmias in the second group are generally described as hæmolytic, but such a description implies that they are the result of destruction of the circulating red cells (hæmolysis), and whereas this may sometimes be the case, at other times the precursors of the red cells in the hæmatopoietic centres may be damaged or even destroyed. Hæmolysis may, and probably usually does, accompany such damage to the hæmatopoietic centres, but theoretically it is possible that the injury may be limited solely to the hæmatopoietic centres. For these reasons it seems wiser to give this group of anæmias a name which suggests that the whole or any part of the erythron may be attacked, and it is for this reason that we have introduced the term ' erythronoclastic.'

Although at first sight these two groups seem to be quite sharply differentiated, a closer acquaintance with the anæmias of infancy shows that it is sometimes difficult to determine in any particular case if the anæmia arises solely as a result of a deficiency of some factor essential for hæmatopoiesis or as a result of destruction of some part of the erythron. For instance, it is possible for an anæmia to be produced as a result of abnormal destruction of the erythron, but to be continued as a deficiency disease; in other words, the factor which causes the anæmia may differ from that which prolongs it. This state of affairs is perhaps best exemplified in the anæmia of prematurity. Then again the effects of infections upon the erythron may further complicate and obscure the picture; thus, an infection may be responsible for severe or mild hæmolysis, which in its turn may be engrafted on a deficiency anæmia. Again, not only may hæmolysis occur in the course of a nutritional anæmia, but there is clear evidence that in many instances the hæmatopoietic portions of the erythron also suffer damage, as is shown by the fact that in infections the beneficial effect of iron is greatly diminished, or even absent. In this way a premature child suffering from anæmia may have developed anæmia as a result of prolongation of the hæmolysis which normally occurs at birth; may continue to be anæmic from a nutritional deficiency; and may become more anæmic as a result of infection and may be resistant to treatment by iron until the infection is removed. In such a case the classification of the anæmia and the unravelling of the mechanism of its production would present considerable difficulties; indeed, one writer has gone so far as to say that a case of chronic anæmia due to a single ætiological factor is a great rarity.

Josephs[11] has attempted to investigate the mechanism of production of anæmia in children by estimating the amount of urobilin in the urine. He regards the ratio of the ' urobilin ' excreted per day to the total circulating hæmoglobin as a measure of the amount of blood destruction which is taking place. In the term ' urobilin ' he includes all the substances giving Elman and McMaster's reaction for urobilin, and he defines a unit of blood destruction as the daily excretion of 0.4 mgrm. ' urobilin ' per gramme of circulating hæmoglobin. Assuming that the blood volume is 76 c.cm. per kgrm., the amount of circulating hæmoglobin is obtained from the formula Wt. (in kgrm.) × Hb (grm. per 100 c.cm.) × 0.76. If the hæmoglobin is stationary the units of blood destruction and formation, when calculated in this manner, are equal. This method of investigation failed in premature children during the first four months of life, but was useful for ' post-infectious anæmia.' In the anæmias produced by infection ' urobilin ' was found to be increased. Sometimes such a rise was found without infection, but this was not due, as has been suggested, to some toxic substance in milk, e.g., fat, because without any change in diet the cells and hæmoglobin might increase and then the ' urobilin ' decreased.

The condition known under the various names of von Jaksch's anæmia, splenic anæmia of infants, anæmia pseudo-leukæmica infantum, is difficult to place in any classification. In the past this group of anæmias has been a medley, but in our opinion the condition known in this country as von Jaksch's anæmia is really a form of hæmolytic anæmia, and we have therefore dealt with it under that head. From time to time there have been described in the literature instances of anæmia occurring in the new-born child and described as congenital anæmia or primary anæmia of the new born. Some of these fall into the category of the hæmolytic (erythronoclastic) anæmias, whereas others are deficiency diseases and due to defective ante-natal storage.

Aplastic anæmia is extremely rare in infancy. It is recognized by the complete inability of the marrow to reproduce any of its three series of blood cells, erythron, granular leucocytes, blood platelets. Its ætiology is as yet undetermined, although its occurrence is sometimes regarded as the result of a congenital tendency to marrow aplasia. There is, however, no evidence justifying this view to the exclusion of the possibilities that it is either a deficiency disease affecting early myeloid cell development, or a primary destructive disease of all the marrow series, erythron, thrombocyte, and myeloid leucocyte system, which has completely and irrevocably paralysed the bone marrow. In the cases we have observed the evidence was in favour of the latter view, under which head they have therefore been classed.

Sickle-cell anæmia and acholuric jaundice are regarded by Boycott as congenital malformations of the erythron. Finally, continental writers have described an anæmia which develops in some babies who are fed on goat's milk. Goat's milk anæmia is characterized by the presence of megaloblasts in the blood stream and is probably a deficiency anæmia possibly allied to the megalocytic form of deficiency anæmia occasionally found in cœliac disease, and which is described in detail in a later paper (Part III).

Leukæmia.—Leukæmia is not, of course, a disease of the erythron, but it is impossible adequately to consider the anæmias of childhood without including a survey of leukæmia, for at least three reasons: (1) the mechanical or toxic effect on the erythron produced by leukæmia is so marked that a severe grade of anæmia is produced; (2) the light thrown by a careful study of the cytology of the blood and hæmatopoietic centres on the question whether extra-medullary centres are functioning as either leucopoietic or hæmatopoietic foci, or both; (3) the help afforded by the correlation of the cytological features of the peripheral blood with those of the tissues in assessing the rôles played by the various blood cells. For these reasons we have included in our studies all the cases of leukæmia seen in the last three years on which an autopsy has been performed.

Methods.—Hæmoglobin has been estimated by Haldane's method in which 100 per cent. represents 13.8 grm. of hæmoglobin per 100 c.cm. of blood. The

instruments used were standardized against those used by Price-Jones[12] with which he found the mean percentage of hæmoglobin in 100 healthy men to be 105 and the range 96 to 116, and in 100 healthy women a mean of 98 and a range of 90 to 110. The samples of blood giving these figures were taken by pricking the skin, a method which has been adopted by us. Dr. Helen Mackay informs us that on comparing her hæmoglobinometer with the Price-Jones standard there is a difference of 7 per cent.; 107 per cent. on the Price-Jones standard corresponding to 100 per cent. on her instrument.* This difference should be borne in mind when comparing our results with those obtained by her, and explains why the infants who recovered from nutritional anæmia gave a hæmoglobin of 84 per cent. whereas in her cases the value on recovery was 80 per cent. The other methods used have been those ordinarily employed in the clinical investigation of anæmia, and in addition, during the last four months Price-Jones curves have been constructed in a fair number of instances and estimations of cell volume, etc., made by the hæmatocrit.

Acknowledgments.—In these studies of anæmia I have had the privilege of being associated with a band of enthusiastic workers and the papers that follow are the result of their efforts; all have had a finger in the pie, and it is left to others to decide whether anyone has pulled out a plum. Of those who do not appear in the rôle of author, special thanks are due to Dr. Carey Smallwood, who has been responsible for all the Price-Jones curves, and to Miss Finch, on whom has fallen a large part of the hæmatological examinations in connection with the rat colony. We have received much encouragement and help from our colleagues at the Children's Hospital and in the Child Welfare Service of the City of Birmingham, who have freely placed their cases at our disposal, and for this we are grateful. We also place on record our indebtedness to the nursing and technical staffs of the hospital for much hard work graciously performed. Dr. Hugh Ramage of Norwich has kindly carried out spectrographic examinations for the presence of metals in certain yeast preparations, and for this we tender him our best thanks. The drawings and micro-photographs are the work of Mr. E. B. Brain, the hospital artist. Finally, we acknowledge with gratitude the help received from the Medical Research Council, who have not only made a personal grant to Dr. E. M. Hickmans, but also have defrayed the expenses incurred in this research.

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* A new paper on this subject by Dr. Mackay will appear in the next number.

Part II.—The effect of yeast on nutritional anæmia in rats

BY

LEONARD G. PARSONS, M.D., F.R.C.P.,

AND

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The literature upon experimental nutritional anæmia has assumed such proportions that a complete review of it is quite outside the scope of this paper; nevertheless there are certain outstanding and historical contributions which must be considered if a discussion of the problems raised in this and the succeeding papers are to be at all intelligible.

In 1889 Bunge[1] found that the amount of iron present in the liver and spleen, and the total amount of iron in the body expressed as a percentage of the body weight, were highest at birth, and thereafter fell progressively until they reached a minimum at the end of the lactation period. He also held that iron was absorbed from the alimentary canal only when in organic combination in the food, and that the presence of an excess of inorganic iron produced an increase in the absorption of food iron. Ten years later his pupil, Abderhalden[2], produced nutritional anæmia in animals by limiting their diet to milk for a considerable period after the end of the lactation period, and found that the addition of inorganic iron to diet of these animals did not bring about an increase in hæmoglobin. His comment on this result was as follows: The mere fact that the addition of iron to nutriment poor in iron does not have any distinct influence on the formation of hæmoglobin . . . indicates that other building material is wanted as well as the iron. He also proved the inaccuracy of the statement that inorganic iron could not be absorbed from the bowel.

From this time no experimental observations were made which had important bearing on nutritional anæmia until in 1920, Whipple and Robscheit-Robbins of Rochester, New York State, commenced an investigation on the rate of hæmoglobin formation in dogs previously rendered anæmic by repeated bleedings and which were given a diet low in iron. These researches led to a renewal of interest in the effect of iron on hæmoglobin formation, and in 1925, Hart, Steenbock and their colleagues[3] at the University of Wisconsin started on what has proved to be the most important and extensive work on experimental nutritional anæmia. Amongst other things they found that impure salts of iron cured nutritional anæmia, but that pure iron salts were quite ineffective: further, that to obtain a cure the addition of copper to iron salts was essential. These results have been challenged along two main lines. First, Drabkin and Miller[4] state that the exhibition of certain amino-acids which they claim to be copper-free will cure nutritional anæmia. Secondly, Beard[5] and his co-workers, although agreeing that copper is the most effective curative supplement to iron, state that other metals, nickel, germanium, manganese, arsenic, vanadium, titanium, zinc, rubidium, chromium, selenium, mercury, can also act as curative supplements; and that the addition of pure iron alone to a milk diet will effect a cure. These criticisms have

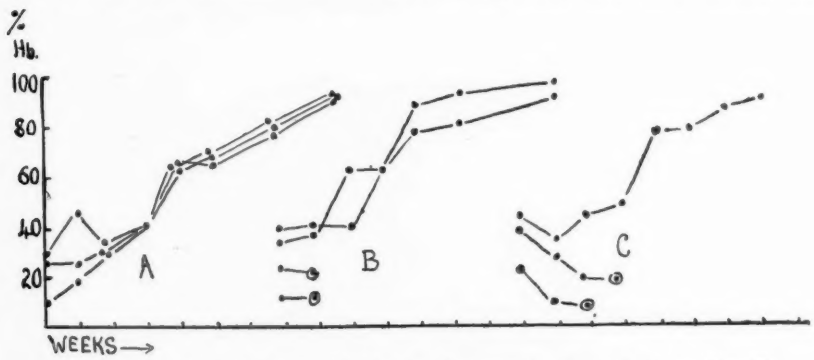
been answered, apparently satisfactorily, by the Wisconsin workers, for the balance of opinion strongly supports them. Their latest contribution (Elvehjem and Sherman[6]) shows that in the absence of copper, inorganic iron is readily assimilated by rats suffering from nutritional anæmia and stored in the liver and spleen, but that it cannot be used until copper is supplied, when the greater part of the iron in the liver is removed and built into hæmoglobin.

Preliminary experiments with yeast.—Some two years ago we decided to try the effect of adding yeast to the diet of black and white rats which had been rendered anæmic by prolonged milk feeding. One gramme of a dried preparation of brewer's yeast (Yestamin, of the English Grains Company) was therefore added to the daily milk ration of each rat, and it was found that thereafter the hæmoglobin rose steadily until a normal value was attained. Further, unlike control rats cured by copper and iron, these rats reproduced within a relatively short time after the anæmia was cured, although at the end of the lactation period (21 days) the progeny was found to be anæmic: in the individual members of the litter the hæmoglobin ranged from 12 to 25 per cent., and some also showed splenomegaly. These results were so surprising that we decided to repeat them, and to attempt to find what curative factor or factors were present in yeast.

The details of these preliminary experiments may now be given. The rats used were rendered anæmic by feeding them on liquid milk ad lib., and when two months old their hæmoglobin ranged from 10 to 45 per cent. These were then divided into four groups, which were fed as follows:—

Group.	Diet.	Supplement (daily).	% Hb. in each member of group.
A.	Liquid milk.	1 grm. (about) dried yeast ...	12, 27, 34
B.	Liquid milk.	Ditto +0·005 mgrm. Cu	10, 24, 34, 39
C.	Liquid milk.	0·5 mgrm. Fe (pure $FeCl_3$) ...	25, 42, 45
D.	Liquid milk.	Nil.	Died.

The only group in which all the rats recovered was the yeast group (Group A), the hæmoglobin of these rats reaching over 80 per cent. in a period of from six to seven weeks. One rat in the pure iron group (Group C) achieved normal hæmoglobin figures, and two died; but all



GRAPH I.—Rise of hæmoglobin in Groups A, B and C, on liquid milk with various supplements (see text). ⊙ died.

the control rats (Group D) died in a few days. Comparisons of the rate of hæmoglobin formation in Groups A, B, and C, are shown in Graph 1.

Waddell, Steenbock and Hart[7] found that growth and reproduction were below normal on a diet of whole milk and copper, although eventually they succeeded in obtaining some litters, very few of which were nursed; also that on milk, copper and iron, reproduction was still subnormal and there was poor rearing of the young. The doe in the Group A, i.e., on a diet of milk and yeast, when about seven months old produced a litter, which she consumed; eventually she had two further litters, the intervals between them, however, being about three months. An account of these two litters, here designated A and B, is appended.

Litter A.—The first surviving litter was not examined for three weeks after birth. The young rats were apparently normal in activity and growth as far as could be judged without handling them, but when examined on the 21st day they were found to be pale and slightly under normal weight; the average weight being 27.5 grm. against a normal weight at this age of 30 to 35 grm. One rat selected at random from the litter showed hæmoglobin of 15 per cent. only, and on post-mortem examination the organs were found to be anæmic and the spleen somewhat enlarged. After removal of the intestinal tract and small portions of the organs for microscopical examination, the iron and copper content of the rat was estimated. The iron content was found to be low but the copper content was very similar to that of a stock rat of the same age. The figures are as follows:—

Body weight	Anæmic rat 22·20 grm.	Stock rat 30·80 grm.
Percentage Fe (dried weight) ...	,, ,, 8·48 mgrm.	,, ,, 16·80 mgrm.
,, Cu ,, ,, ...	,, ,, 0·82 ,,	,, ,, 0·90 ,,
Total Fe per rat	,, ,, 0·66 ,,	,, ,, 1·67 ,,
,, Cu ,, ,, ...	,, ,, 0·06 ,,	,, ,, 0·09 ,,

Another rat, which had been noticed to be consuming some of the mother's diet (yeast and milk), was killed on the 23rd day, and blood was taken from the heart immediately after death. The results of the examination of this blood compared with that of a stock rat of the same age were as follows:—

Red cells	3,340,000 ...	Control 5,625,000
White cells	14,600 ...	,, 6,000
Hæmoglobin	12 per cent. ...	,, 75 per cent.
Colour index	0·2 ...	,, 0·67

At the same time a complete examination was made of the mother rat's blood, and this was compared with the blood of a stock rat of the same age, fed on stock diet, two days after weaning her litter of eight. The results were as follows:—

Red cells	5,420,000 ...	Control 7,720,000
Hæmoglobin	95 per cent. ...	,, 98 per cent.
Colour index	0·9 ...	,, 0·6
Polymorphonuclears	63 per cent. ...	,, 50 per cent.
Lymphocytes	31 ,, ,, ...	,, 45 ,, ,,
Transitional	5 ,, ,, ...	,, 2 ,, ,,
Eosinophil	1 ,, ,, ...	,, 3 ,, ,,

The conditions under which the stock rat was chosen make the results strictly comparable and if the details which are given above are consulted it is obvious that the blood in both instances is normal.

One week later for no obvious reason a third member of the litter died, aged 28 days, and in Table 1 are given the weights of the heart,

TABLE 1.

WEIGHT OF ORGANS OF ANÆMIC RAT, WITH CONTROLS.

	Anæmic rat Litter A	Normal rat of same age	Normal rat of same weight
Heart	0·48 grm.	0·22 grm.	0·23 grm.
Liver	2·15 ,,	1·98 ,,	1·2 ,,
Kidneys (2) ...	0·66 ,,	0·54 ,,	0·36 ,,
Spleen	0·32 ,,	0·09 ,,	0·09 ,,
Body weight ...	34·14 ,,	43 ,,	32 ,,

liver, kidneys and spleen, and for comparison the weights of these organs in normal rats, fed on stock diet, of the same age and weight. From the results it is obvious that all these organs, and the spleen in particular, show enlargement, an enlargement which is all the more marked when it is considered in relation to the body weight. This rat had been taking yeast and milk for one week before death.

LITTER B.—The second surviving litter was born three months after litter A; in view of the results obtained in the former litter this one was watched more carefully and examined earlier. The members of the litter made excellent progress, and, although pale, were very active. One rat was killed when four days old, and blood immediately removed from the heart for examination. The blood picture showed a definite anæmia when compared with average figures from six stock rats of the same age:—

Red cells	4,060,000 ...	Control	3,162,000
White cells	8,000 ...	,,	4,230
Hæmoglobin	52 per cent. ...	,,	78 per cent.
Colour index	0·65 ...	,,	1·2

Some of the organs of this rat were weighed and found to be of much the same weight as those of a stock rat of the same age, but relatively heavier in relation to the body weight. It is obvious, therefore, that the organs of the anæmic rat from litter B were larger than normal.

Another rat of this litter was killed at 21 days, and a pronounced anæmia was still present. The hæmoglobin and colour index were 25 per cent. and 0.3 respectively, as compared with 72 per cent. and 0.8 in controls. The anæmia was not quite so marked as in litter A. In this particular rat the viscera were slightly under normal weight.

The remaining rats of both litters A and B received various forms of treatment for their anæmia (p. 104).

Doe of Litters A and B.—It is not possible to give accurate figures of the amount of yeast consumed by this doe in the course of these experiments, because much of it settled at the bottom of the dish of milk during the day and was not eaten. Presumably during her second pregnancy (litter A), which occurred whilst she was still growing, she managed to obtain a sufficient supply of iron to maintain a normal amount of hæmoglobin during growth, although insufficient to supply the fœtuses with their full requirements. It is clear that she did not sacrifice her own iron storage to supply the fœtuses. There were only two possible sources from which she could obtain iron, her diet and her first litter which she had consumed; the latter, judging from the results obtained in later litters, could not have been a very fertile source of iron. During the third pregnancy (litter B), when she was fully grown, she required less iron for her own maintenance, and the resulting slight excess over her own requirements was available for the fœtuses; these, therefore, eventually showed a somewhat higher hæmoglobin than those of litter A.

In a later experiment we attempted to prove the truth of the foregoing explanation by adding dried yeast to the milk diet of a congenitally anæmic rat, together with pure iron, prepared according to the directions given by Steenbock, Waddell and Hart.[8] This diet was given continuously from the time the rat was weaned and throughout pregnancy. The resulting litter did not show anæmia and actually at the end of lactation the young rats had hæmoglobin values which were greater than normal. This experiment, we maintain, bears out the view that yeast in the quantities given, and milk, do not during the periods of pregnancy and lactation contain the amount of iron necessary to produce normal litters.

Further experiments in production of anæmia of the new born in rats.—Since this preliminary experiment we have obtained a number of litters from rats fed on milk and yeast. The hæmoglobin percentages of the members of these litters at 21 days have varied from 12 to 75 with an average of 38; the highest figures may be due to the fact that some of the rats were consuming considerable amounts of their mother's diet (yeast and milk) before they were 21 days old. The hæmoglobin values of different members of the same litter were found to vary a great deal, just as at times occurs with human twins or triplets. Estimations made on a few rats at the age of four days showed a range of hæmoglobin from 52 to 85 per cent., compared with a range of 65 to 85 per cent. in our stock rats at the same age. After the rats were a week old it was usually quite easy to see that they were definitely pale when compared with stock rats of a like age, and indeed the profound anæmia exhibited by these young rats is remarkable. Scott[9] obtained from does which were fed on white bread and milk from puberty to ten months of age and which 'showed practically no anæmia themselves' litters which, when given the same diet, developed a hypochromic anæmia with a tendency

to spontaneous cure as they grew older. In contrast to these results, we have described instances of rats which suffered from nutritional anæmia, were cured by a yeast and milk diet, and then produced young with a pronounced anæmia although they themselves remained normal. Further, as will be shown later on in this paper, this anæmic progeny can be cured by the same diet, or a normal litter can be obtained from their mother by adding 0.5 mgrm. of pure iron (as ferric chloride) daily to her diet of milk and yeast.

For the purpose of experimental work on the cure of nutritional anæmia we have made use of many rats bred from milk-and-yeast fed mothers, and we now always reduce their hæmoglobin to a very low value before commencing any experiment. We can reduce the hæmoglobin of such rats to 10 per cent. by feeding on milk only for fourteen days after weaning, whereas rats from parents fed on stock diets require six to eight weeks' milk feeding after weaning to reduce the hæmoglobin to 20 per cent.; indeed in some cases we have been unable to reduce the hæmoglobin below 30 to 40 per cent. after three months of feeding on a diet consisting only of milk. Since these preliminary experiments, we have not made use of rats for testing the curative value of the various methods of treating nutritional anæmia, unless their hæmoglobin had been reduced to less than 30 per cent.

Another interesting thing has been noted in the course of these experiments, namely, that when these piebald rats become anæmic the darkly pigmented hairs lose much of their colour, but that this is restored as the anæmia progresses towards cure, although the rate of progress is not the same in all cases. It has been suggested to us that the anæmia of new-born rats might be due to infection with Bartonella muris; search has therefore been made on several occasions for evidence of infection by this micro-organism but always with negative results.

These results appear to demonstrate quite definitely that the pre-natal storage of iron in the progeny of milk-and-yeast fed rats is deficient even in those cases where the hæmoglobin soon after birth is fairly high.

Curative factor or factors in yeast.

In view of the results of these preliminary experiments attempts have been made to find out what it is in yeast that is responsible for the cure of nutritional anæmia. This is a deficiency anæmia and the deficiency is in one of the substances, iron, copper, Vitamin C, thyroxin, which are necessary for the maturation of the normoblast in the red blood cell. For its cure two processes are essential: (1) the production of new red cells measured by the degree of reticulocytosis and leading to an increase in red cells; a process sometimes referred to as hæmatopoiesis; (2) the synthesis of hæmoglobin. It is clear that hæmoglobin cannot be put into red cells unless a supply of these cells is available; also that unless material for the synthesis of hæmoglobin is available, cure is impossible

even if the supply of red cells is ample. It is conceivable that two factors are necessary for these two processes or that the same factor may initiate both; thus, in the case of iron, Vitamin C and yeast we have evidence showing that they are capable of initiating both these processes, whereas copper is of use only in hæmoglobin synthesis.

Composition of yeast. — Yeast is rich in the Vitamin B complex, contains iron both in inorganic and organic form, copper, manganese, and in brewer's yeast a trace of arsenic, also two proteins zymocasein and cerevisine. The organic iron in yeast is of considerable interest. According to Warburg[10] respiration in most living cells is due to a process catalysed by iron, this respiratory enzyme being a hæmatin compound. Actually more than forty years ago MacMunn[11] of Wolverhampton found that a pigment related to hæmoglobin (myohæmatin), which he believed to be respiratory in function, occurred in the cells of animals. This pigment has been extensively studied by Keilin[12] during the last eight or ten years and has been renamed by him ' cytochrome.' He has shown that it is present in plants; that it is widely distributed in the cells of ærobic, but not anærobic, micro-organisms; and that its highest concentration is found in cells capable of active metabolism, such as the thoracic wing muscles of flying insects, heart muscles of mammals and birds, pectoral muscles of flying birds, yeast and some bacteria, particularly the diphtheria bacillus. Cytochrome exists in two forms: (1) oxydized, from which (2) the reduced form is produced by the reducing action of the tissues in the absence of oxygen. In the reduced form it has a very characteristic absorption spectrum composed of four mains bands *a*, *b*, *c*, *d*. Cytochrome is not a single compound but is a mixture of three hæmatin compounds (hæmochromogens) a^1, b^1, c^1, each of which shows two characteristic bands alpha and beta, the alpha bands of each corresponding respectively with the *a*, *b* and *c* bands of cytochrome, whereas the beta bands are the components of the *d* band of cytochrome. Cytochrome acts in the tissues as an oxygen carrier from which they obtain oxygen by reduction, whilst it spontaneously re-oxydizes by absorbing oxygen from its surroundings, that is, it is autoxidizable. Of the three components of cytochrome it is only the b^1 component which is autoxidizable; the components a^1 and c^1 are not. Keilin has further shown that the cells of ærobic organisms also contain an unbound hæmatin similar to the protohæmatin of hæmoglobin. This unbound hæmatin is also autoxidizable so that two out of the four hæmatin compounds in the cell are autoxidizable. Iron is therefore present in yeast, in four organic compounds, all of which are closely allied to hæmoglobin. Recently Stone and Coulter[13] have obtained from yeast and also from B. phosphorescens and C. diphtheriæ by acetic acid-ether extraction hæmatin and a complex porphyrin which on disintegration yields coproporphyrin and the copper compound of porphyrin.

The proteins of yeast were shown by Thomas[14] to consist of zymocasein a phospho-protein resembling casein, and cerevisine a yeast

albumin. Both these proteins have been broken up into their constituent amino-acids; zymocasein by Löwers and Nowak[15] and cerevisine by Meisenheimer[16] with the following results:—

Amino-acid.					% of total nitrogen of cerevisine.		% of total nitrogen of zymocasein.
The	nitrogen	of	Glycine	forms	0·5	and	7·4
,,	,,	,,	Alanine	,,	10 to 15	,,	1·7
,,	,,	,,	Valine	,,	10 to 15	,,	0
,,	,,	,,	Leucine	,,	5 to 10	,,	3·2
,,	,,	,,	Phenylalanine	,,	8	,,	0·9
,,	,,	,,	Tyrosine	,,	2	,,	2·4
,,	,,	,,	Cystine	,,	2	,,	0·7
,,	,,	,,	Proline	,,	2	,,	4·0
,,	,,	,,	Glutamic acid	,,	6	,,	19·5
,,	,,	,,	Asparagine	,,	3·5	,,	present
,,	,,	,,	Tryptophane	,,	0·5	,,	1·5
,,	,,	,,	Histidine	,,	0	,,	3·4
,,	,,	,,	Lysine	,,	10	,,	11·5
,,	,,	,,	Arginine	,,	10	,,	8·4
,,	,,	,,	Purine	,,	12	,,	0·6
,,	,,	,,	Melanine	,,	0	,,	2·3
,,	,,	,,	Ammonia	,,	8	,,	9·3

Drabkin and Miller[4] have reported the cure of nutritional anæmia in rats by the addition of certain amino-acids to a milk diet, the most effective amino-acids being arginine and glutamic acid and its salts. It is interesting to note that of all the amino-acids of yeast glutamic acid is present in the largest amount and that lysine and arginine follow in that order. In assessing whether or not these acids are of importance in hæmatopoiesis or hæmoglobin-building, it must also be remembered that Elvehjem, Steenbock and Hart[17] have demonstrated that if glutamic acid is properly purified it is completely inactive as a supplement to iron.

We thought at first that the curative action of yeast might be due to its copper and iron content, and therefore we attempted to obtain a preparation of yeast with the lowest possible iron, copper and manganese content.

Anæmic yeast.—A sample of ordinary brewer's yeast was subcultured several times through wort, agar and peptone until a reasonably pure growth was obtained. From this growth a medium, made up of doubly distilled water containing sucrose and the following salts: potassium chloride, ammonium chloride, disodium hydrogen phosphate, calcium chloride, and magnesium sulphate (B.D.H., A.R. products), was inoculated. In this medium, which was aerated by the continual suction of filtered air through it, growth was very slow, but gradually the yeast was deposited at the bottom of the medium in a very fine white condition. The yeast was subcultured every three or four days and finally, after separating it from the medium by centrifugalization. was washed with doubly distilled water. Yeast so obtained differs from ordinary yeast in

several ways. Its colour is white and not the normal brownish colour; indeed, as stated by Elvehjem[18] who independently obtained a similar variety of yeast, this yeast may be regarded as itself anæmic and for the purpose of reference in this paper it is called 'anæmic' yeast. Its morphology is also altered and it takes on a mycelial form; indeed some people to whom we have shown this anæmic yeast are doubtful if it really is yeast. We have, however, succeeded in returning it to almost a normal appearance by subculturing five or six times in the same medium described above with the addition of copper and iron (Fig. 1 and 2). Elvehjem has shown that anæmic yeast, prepared by this method, is free

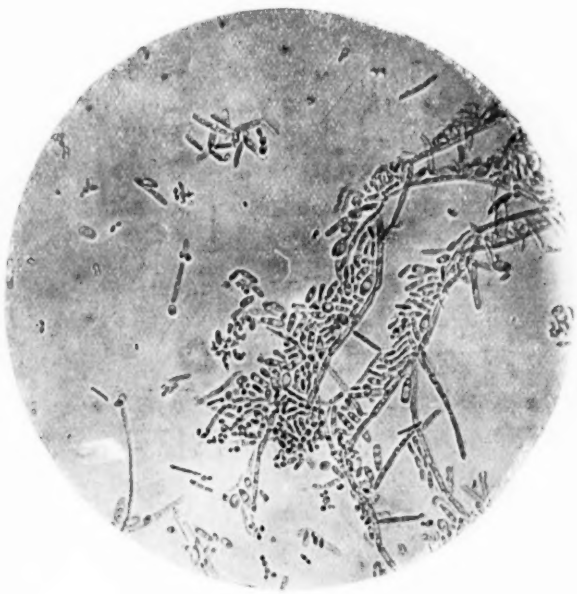


FIG. 1.—Microphotograph of 'anæmic' yeast shewing mycelial forms.

FIG. 2.—The same yeast after 6 sub-cultures into the same medium + Fe. 0·5 mgrm. and Cu. ·05 mgrm. per 100 c.cm. (approx.).

from inorganic iron, the total iron content being only one-third to one-half of the normal; that the copper content is exceedingly low; and that the addition of a small amount of iron to the medium accelerates the growth of the yeast and increases the cytochrome content to normal. He also found that the addition of both iron and copper to the medium gave a further increase in cytochrome and caused the production of cytochrome containing a distinctly higher content of the a^1 hæmochromogen showing that copper had a specific effect on this component.

Cure of anæmia by anæmic yeast.—Tests of this anæmic yeast were carried out on some members of litter A to which we have referred previously. The rats were kept in cages made entirely of glass to prevent

the possible fallacy of their obtaining iron or other metal from the material of which the cages were made. In the earlier experiments many of the very young rats became œdematous, but when a dried milk powder was substituted for liquid milk the œdema disappeared. Whether or not this was due to the alteration in the type of food cannot be stated because liquid milk has been used in some later experiments without the development of œdema. Distilled water has been given for drinking purposes.

In order to compare the effects of treatment with anæmic yeast with that of other forms of treatment the litters were divided into groups.

LITTER A.—The anæmic rats of this litter, with hæmoglobin reading of 12 to 25 per cent. at weaning, were divided into four groups and fed as follows:—

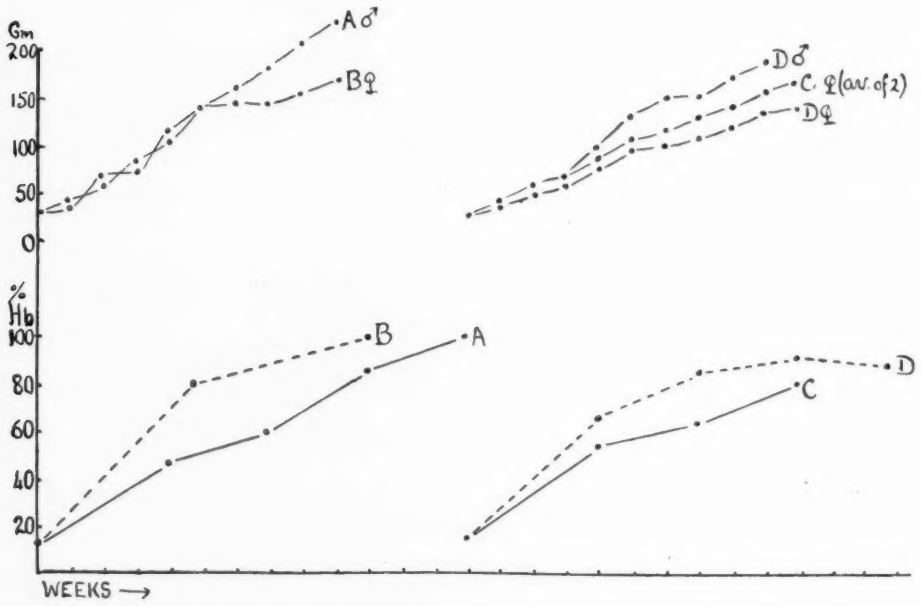
Group	(A)	Dry milk powder + 1 grm. dried yeast per rat	1 buck
,,	(B)	Dry milk powder + 1 grm. dried yeast + 0·5 mgrm. pure Fe. per rat	1 doe
,,	(C)	Dry milk powder + about 0·3 grm. anæmic yeast per rat ...	2 does
,,	(D)	Dry milk powder + about 0·3 grm. anæmic yeast + 0·5 mgrm. pure Fe. per rat	1 buck 1 doe

LITTER B.—Those in litter B were fed in two groups:—

Group (E) Dry milk powder + liver extract

,, (F) Dry milk powder + liver extract + 0·5 mgrm. pure Fe. per rat

The progress made by the rats in litter A is shown in Graph II and Table 2, and by those in litter B in Graph III, and on the latter graph



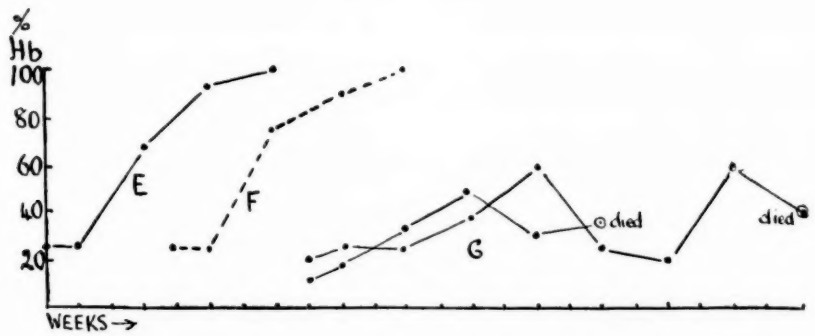
GRAPH II.—Litter A. Weight and hæmoglobin curves of Groups (A) to (D).

are added for comparison two graphs of the hæmoglobin of rats (G) fed on a dry milk powder only. Since the dry milk powder used in this control was richer in iron than the undried milk used in the experiments described above (p. 96) this control was considered necessary to make sure that milk powder did not contain sufficient iron to account for the rise of hæmoglobin.

TABLE 2.

BLOOD CHANGES IN LITTER A AFTER 7 AND 10 WEEKS ON VARIOUS DIETS (SEE TEXT).

Group	a	b	c	d
Hæmoglobin per cent.	60	87	65	85
Red blood cells	6,820,000	7,640,000	6,570,000	8,100,000
Size and shape	normal	normal	normal	normal
Polychromatophilia	present	very slight	present	slight
White blood cells	4,800	6,000	5,000	5,400
Hæmoglobin per cent.	86	95	82	92
Red blood cells	7,080,000	8,100,000	5,260,000	7,210,000
White blood cells	4,500	6,000	7,000	4,500
Reticulocytes per cent.	1·5	10	12—15	5



GRAPH III.—Litter B. Hæmoglobin curves: with curves (G) of two rats fed with dried milk powder only.

From a consideration of Graphs II and III it is clear that:—

(1) The addition of anæmic yeast to milk leads to a cure of anæmia although more slowly than by other methods used in this experiment (Group C).

(2) Additional iron (0.5 mgrm. per rat per day, not all of which was consumed) permitted a quicker formation of hæmoglobin than anæmic yeast (Group D).

(3) Dry yeast stimulates hæmoglobin formation more rapidly than anæmic yeast (Group A).

(4) The addition of iron to yestamin increased the rate of hæmoglobin formation (Group B).

(5) Liver extract raises the hæmoglobin quickly and here also the rate is increased by the addition of iron (Groups E and F).

The facts that nutritional anæmia of rats can be cured by adding yestamin to the milk diet, and that it can be cured, though more slowly, by adding anæmic yeast to the milk diet, have now been confirmed on many animals.

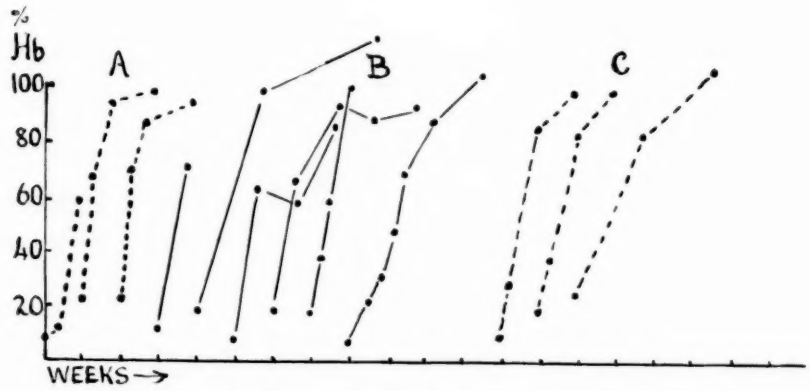
The daily ration of each rat fed on milk and yestamin is 10.0 grm. of dried milk and 1.0 grm. of yestamin. The iron in different samples of yestamin varies from 20.5 to 43.1 mgrm. per cent., but 1.0 grm. of the yestamin used in these early experiments contained 0.25 mgrm., an amount which according to Beard and Myers[5] probably represents the minimum daily iron requirement as an addition to a whole-milk diet for the cure of young growing rats suffering from nutritional anæmia.

The amount of copper in yestamin also varies, but within narrower limits. We have found values from 2.45 to 3.13 mgrm. per cent. In these experiments the amount of copper in 1.0 grm. of yestamin was 0.025 mgrm., which according to Beard and Myers is the minimum effective daily dose required as a supplement for 0.5 mgrm. of iron, but the amount of manganese present in this specimen of yestamin was 0.003 mgrm. which is considerably less than the minimum daily supplement (0.1 mgrm.). It is therefore clear that if all the yestamin and milk rations were consumed, which as a matter of fact did not happen, there was just sufficient iron and copper to cure the anæmia; nevertheless this is not the whole explanation because anæmic yeast in which the total iron content was reduced to 0.13 mgrm. per grm., and the daily dose of which was only 0.25 to 0.33 grm., also produced cure. If the whole of the ration of anæmic yeast were eaten the maximum amount of iron consumed in addition to that present in the milk would be only 0.0425 mgrm.

We have already mentioned that according to Elvehjem anæmic yeast is free from inorganic iron, so that if the cure of anæmia by anæmic yeast is due to its iron content it must be due to the hæmatin compounds of which a description has been given. Elvehjem[19] has carried out a series of experiments to determine the effect of hæmatin on nutritional anæmia which showed that, in the absence of copper, hæmatin was as ineffective as pure ferric chloride, but that in the presence of copper it produced a partial cure of the anæmia, the regeneration being neither so rapid nor so complete as that obtained when inorganic iron (ferric chloride) was used as the source of iron. His results also suggested that all iron compounds must be broken down into iron salts before they can be assimilated, which led him to the important conclusion that the limitations of organs like the liver to act as a therapeutic source of iron, especially if the anæmia is a specific iron deficiency, must be borne in mind. The well-known inefficacy of liver preparations as the sole

treatment in nutritional anæmias is thus explained, and is due to the fact that the hæmatin compounds which form their large organic iron content are not readily broken down in the alimentary canal. It is probable then that the curative effects of anæmic yeast cannot be due, except in part, to its hæmatin content.

By way of contrast to these experiments we have tried the effect of the addition to the milk diet of a combination of iron, copper, and yestamin, and the results produced were astonishing. The amounts added were those used as standards in other experiments, viz.: 0.25 mgrm. of iron, 0.025 mgrm. of copper, and 1.0 grm. of yestamin. The amazing rapidity of the rise of hæmoglobin is shown in Graph IV; and in this



GRAPH IV.—Hæmoglobin curves.
A. Milk powder + Ferrofax powder (·3 grm.).
B. Liquid milk + Ferrofax powder (·3 grm.).
C. Ditto. + yestamin (1 grm.), Fe. (·25 mgrm.) and Cu. (·025 mgrm.).

graph are also shown the effects of the administration of Ferrofax powder (British Colloids, Ltd.) which contains these three ingredients, and, it will be observed, that this preparation also produces an extraordinarily rapid cure.

Anæmic yeast is somewhat lacking in the Vitamin B complex, particularly in B_1. The two does in litter A which recovered on anæmic yeast were mated and continued on this diet. One rat had a litter of five, three being still-born. The two living rats were nursed but were very small and wasted, late in opening their eyes and in developing hair, and altogether very poor specimens. One was killed when 21 days old and blood taken immediately after death showed hæmoglobin 60 per cent., red cells 3,410,000, and colour index 0.9. This was not as low as we expected to find, probably because the rat was so small. (The weights of this rat with controls are shown in Table 3.) The mother's blood on

TABLE 3.

Mother's diet	Anæmic rat	Control same age	Control same weight
	Milk and anæmic yeast	Stock diet	Stock diet
Heart	0·08 grm.	0·18 grm.	0·08 grm.
Liver	1·45 ,,	1·31 ,,	0·50 ,,
Kidneys (2) ...	0·15 ,,	0·43 ,,	0·18 ,,
Spleen	0·01 ,,	0·114 ,,	0·05 ,,
Body weight ...	13·3 ,,	32 ,,	13·4 ,,

the same day showed that the hæmoglobin was only 70 per cent.; this had fallen 10 or 20 per cent. during either pregnancy or lactation, or both. The surviving rat was fostered by a stock doe and lived about five weeks.

Since this time we have fed other rats on dry milk and anæmic yeast, and out of three litters only a very few individuals survived to nine or ten weeks and of these (at least) two developed severe œdema before death. These results suggest that there is a shortage of Vitamin B in anæmic yeast, but not sufficient to show symptoms until it has been fed through two generations.

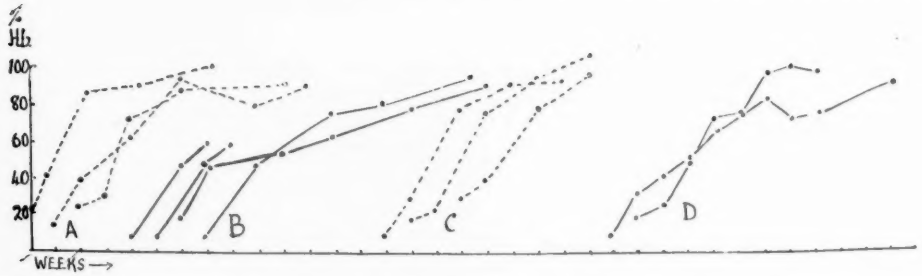
The possibility that the milk used in these experiments with anæmic yeast contained sufficient iron for the cure of anæmia has been considered. Wallgren[20] found that the iron content of milk increased when it was kept in tin vessels; thus, a specimen of cow's milk kept in glass vessels for a period showed an iron content of 0.266 mgrm. per litre, whereas a specimen of the same milk kept for a similar period in a tin vessel contained 0.433 mgrm. per litre. In another experiment milk kept for one week in a glass container contained 0.266 mgrm. and one kept for a week in a tin container 12.25 mgrm. of iron per litre. In his opinion this increase in iron content is chiefly due to the fact that the milk is always somewhat sour so that the lactic and citric acids thus present may attack and dissolve the ferric hydrate, but also there is a possibility of purely mechanical addition of particles of rust. It has been stated by Beard and Myers[5] that when milk is dried by the roller process its iron content is raised by the absorption of iron from the rollers. We have asked the opinion of Mr. A. R. Jephcott, M.Sc., of the Glaxo Laboratories on this point, and he assures us that the amount of iron in dried milk obtained from the source must be infinitesimal, because after many years of use the rollers do not show any pitting. In our opinion the variation in different samples of milk is of greater importance than the possibility of an increase in the iron content brought about by the drying process. The amount of iron in milk taken from different cows may vary as well

as that in different samples from the same cow; for instance, Wallgren in milk from nine different cows found a variation of from 0.140 to 0.320 mgrm. of iron per litre, and exactly the same variation in different samples taken from the same cow. It is possible that different methods of assay account for some of the differing values in iron found by different workers; using the method of iron estimation described by Hart and Elvehjem[21] our figures for the iron content of undried milk have agreed with theirs, and have been fairly constant, but our results with dried milk have varied. For instance, of three samples of milk dried by the roller process, one being produced in New Zealand and the other two in England, we found that the New Zealand milk and one variety of English milk showed higher iron values than the other English milk. This variation in different localities may explain some of the differences between the findings of Hart and his colleagues and those of some other American investigations.

Anæmic rats continued on a diet of undried milk do not live long. On dried milk powder they live considerably longer because this has a higher iron content and also because being a more concentrated diet proportionately more of it is consumed. Nevertheless these animals die before their hæmoglobin becomes normal. In our experience only two rats have ever attained to a normal hæmoglobin value on a milk diet. According to Elvehjem the explanation of this occurrence is that the copper stores have been insufficiently depleted before starting the cure of the anæmia.

Effect on anæmia of watery extracts of yeast containing vitamin B complex, amino-acids and salts.—The next step in the search for the curative factors in yeast was to determine the effect produced by giving a watery extract of yeast, since it would contain the vitamin B complex, amino-acids and certain salts, but no hæmatin. We have made use of two different extracts: (1) marmite, and (2) a preparation made for us by the English Grains Co., which was obtained by extracting brewer's yeast with water and concentrating the extract at a low temperature. For the purpose of experiment 25 grm. of marmite and 25 grm. of yeast extract were each dissolved in 90 c.cm. of water and 5 or 10 c.cm. of the resulting solutions were used for the daily ration of a group of three rats. The amounts actually used were 5 c.cm. of the marmite solution and 5 c.cm. and 10 c.cm. of the yeast extract solution, which meant that each rat in the marmite group had approximately 0.5 grm. of marmite; each rat in the 5 c.cm. yeast-extract group approximately 0.5 grm. of yeast extract; and each rat in the 10 c.cm. yeast-extract group approximately 1.0 grm. of yeast extract. These values are, of course, maximal values because we found it impossible to ensure that all the ration was consumed and equally distributed between the rats, although from the appearance of the graphs it may be assumed that the ration was fairly equally shared.

In assessing results we may assume that a rise of hæmoglobin to 80 or 100 per cent. in six to eight weeks is the normal rate of cure. From a consideration of the Graph V, it is clear that the daily addition of

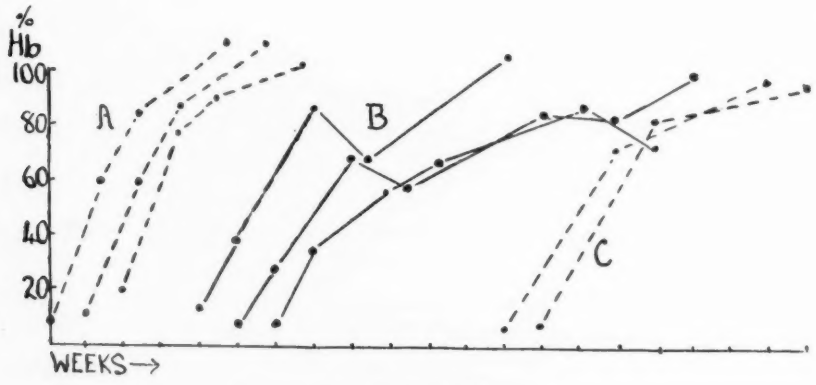


GRAPH V.—Hæmoglobin curves.

A. Marmite ·5 grm.
B. Yeast extract ·5 grm.
C. Yeast extract 1 grm.
D. Yeast residue 1 grm.

1.0 grm. of yeast extract to the diet of dried milk powder brings about a satisfactory rise in hæmoglobin and that the addition of 0.5 grm. of marmite produces almost but not quite as good results. The addition of 0.5 grm. of yeast extract eventually results in a cure, but the time required for cure is much longer than with the larger quantity of yeast extract or 0.5 grm. of marmite and obviously is too small a dose. From these observations it is also apparent that as a hæmoglobin builder marmite is more potent than our yeast extract.

The effect of feeding to anæmic rats the residue left after extracting brewer's yeast to make the special yeast extract was also tested. Again cure resulted, but not quite so rapidly as with the yeast extract (Graph V, D).



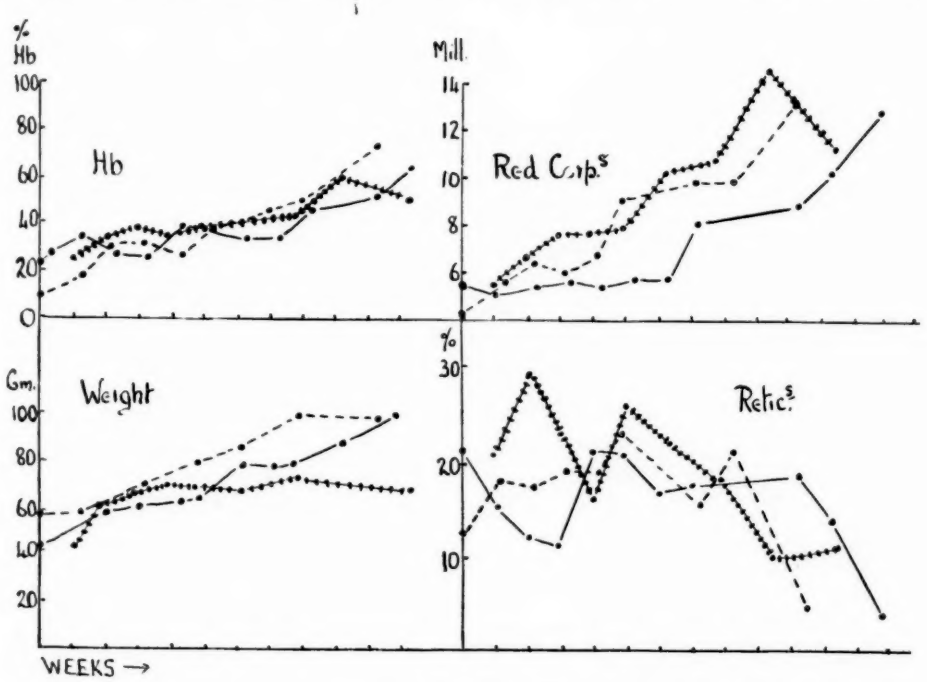
GRAPH VI.—Hæmoglobin curves.

A. Liquid milk + ash from 1 grm. yestamin.
B. Liquid milk + ash from 1 grm. yeast extract.
C. Milk powder + ash from 1 grm. yestamin.

To determine what part vitamin B plays in the cure, yestamin and yeast extract were ashed to destroy Vitamin B, and the ash of each given to separate groups of rats. The ash given to each rat in each

instance is that obtained from the amounts, namely, 1 grm. of yeast extract or 1 grm. of yestamin, which had proved capable of restoring hæmoglobin to normal in six to eight weeks. The ash was dissolved in a slightly acid solution. Actually the yeast extract ashed was from the same sample as that used in the previous experiment; a certain amount of drying had taken place so that the amount of ash given to each rat represents a somewhat larger amount than the extract. The results of feeding these two ashed residues are shown in Graph VI. It will be seen that the yeast extract was rendered rather less potent by ashing, but it cannot be said that ashing yestamin diminished its potency. Curiously enough, the yestamin ash and undried milk was more efficacious than yestamin ash and dried milk powder, a result the opposite to what has usually been found with yestamin itself.

This phase of the problem is now being approached from another aspect. In view of the observation of Wills[22] that vitamin B in the form



GRAPH VII.—Progress of rats fed on liquid milk and vitamin B_2.

of marmite was efficacious in curing tropical macrocytic anæmia, and the probable identification by Strauss and Castle of their extrinsic factor as B_2, we have prepared a vitamin B_1 concentrate from baker's yeast by the method of Kinnersley and Peters, and vitamin B_2 from autoclaved alkaline marmite by the usual method. These are being used for feeding experiments with young stock rats who are placed on a vitamin B deficient diet and then divided into groups, some being given B_1 and others B_2, others again a combination of B_1 and B_2. In addition to

estimation of hæmoglobin and cell counts, Price-Jones curves are constructed, but the experiments have not yet been running sufficiently long to show whether B_2 has any effect on the size of the red cells. In another group of three rats who have been made anæmic (hæmoglobin 10 to 20 per cent.) an arbitrary dose of B_2 prepared from liver extract by Guha's method[23] has been added to the milk diet; in each case a high red cell count has resulted (11 to 14 millions) and the hæmoglobin has risen very slowly to nearly normal level (Graph VII).

Discussion.

From the evidence put forward it is clear that yeast has a curative effect on the nutritional anæmia of rats and allows reproduction to occur. The effect of a diet on growth and particularly on reproduction furnishes a much more stringent test of its completeness than its curative effect on nutritional anæmia, and judged by this standard yeast is a more effective addition to a milk diet than copper and iron, but it is not a complete supplement to the diet because the offspring are anæmic. The addition of yeast and iron to milk does, however, appear completely to rectify the diet in that reproduction occurs and the litter shows a hæmoglobin greater than normal. These examples of anæmia in the new-born rat

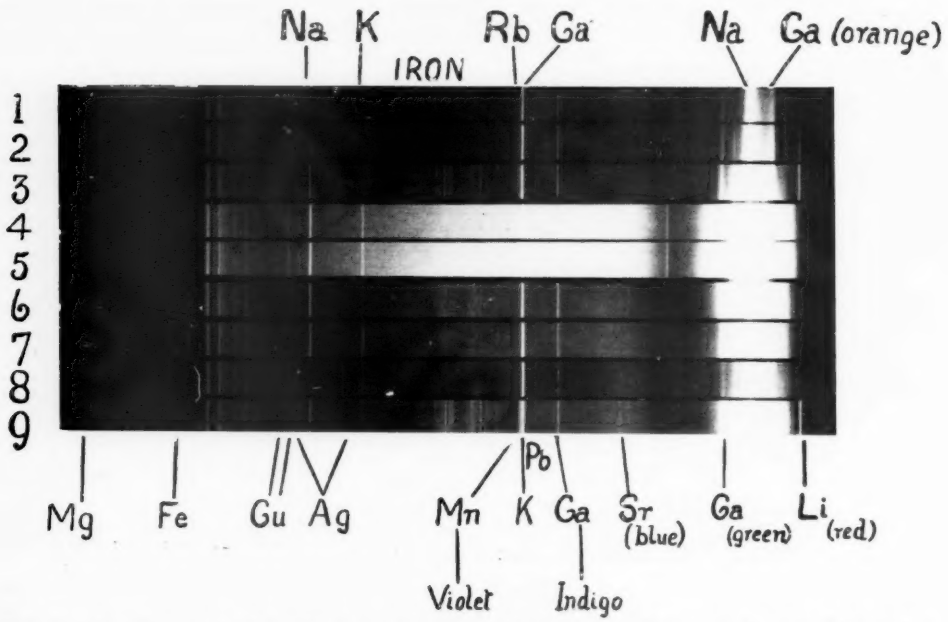


FIG. 3.—Spectrogram of various yeast preparations by H. Ramage, shewing intensity of lines as compared with standard solutions:—

(1) 0·02 c.cm. standard solution.
(2) 0·05 ,, ,, ,,
(3) 0·1 ,, ,, ,,
(4) ·05 grm. marmite.
(5) ,, ,, yeast extract.
(6) ·05 grm. yeast residue.
(7) ,, ,, yestamine.
(8) ,, ,, 'anæmic' yeast.
(9) ·02 c.cm. standard solution.

The standard solutions used have the following percentage composition:—Na. 0·15, K. 0·20, Ca. 0·02, Li. 0·004, Rb. 0·004, Mg. and Fe. 0·05, Cu. and Co. 0·005, Mn. and Ni. 0·002, Cd. 0·10, P. 0·50, Sr. 0·002, Ag. 0·0025 and Pb. 0·05.

appear to us to be strictly comparable to nutritional anæmia of the new-born infant, because both show the characteristics of a nutritional anæmia and can be cured by the same therapeutic method as other forms of nutritional anæmia. Such conclusions seem to be clearly cut and beyond criticism, but when we strive to draw deductions from the experiments designed to discover the curative factors in yeast it is obvious that there are many difficulties.

The part played by iron and copper in these experiments is not easy to assess. We have used the method of Hart and Elvehjem for estimating iron and our results with regard to liquid milk and some samples of dried milk have been consistent with theirs. This method has, however, given values for iron, particularly in marmite and yeast extract, which are completely at variance with spectrographic estimations very kindly carried out for us by Dr. Hugh Ramage. Fig. 3 is a spectrogram of 0.05 grm. of the various preparations we have used, compared with three strengths of the ' standard solution.' The constitution of the ' standard solution ' is indicated in the legend of Fig. 3, and the mineral content of the various preparations as estimated by Dr. Ramage are given in Table 4, where they are compared with the figures obtained by us by microchemical methods (Hart and Elvehjem's method for iron).

TABLE 4.

COMPARISON OF ESTIMATION OF METALS IN VARIOUS YEAST PRODUCTS BY SPECTROGRAPHIC AND MICRO-CHEMICAL METHODS, PARTS PER CENT. IN DRY MATERIAL.

Method	Spectrographic								Micro-chemical	
Metal	Na.	K.	Mg.	Ca.	Mg.	Cu.	Fe.	Ni.	Fe.	Cu.
Marmite ...	high	high	0·02	trace	none	0·008	none[1]	?	0·043	0·004
Yeast extract	high	high	0·03	trace	none	0·02	none		0·032	0·020
Yeast residue	0·60	0·60	0·20	0·08	0·008	0·01	0·03		0·039	0·009
Yestamin ...	0·60	1·15	0·05	0·04	trace	0·003	0·015		0·026	0·003
Anæmic yeast	trace	1·18	0·07	0·008	trace	0·005	trace	trace	0·013	0·003

[1] Another examination showed ' trace.'

Elvehjem states that anæmic yeast is poor in copper, and is free from inorganic iron, and that its total iron content is one-third to one-half that of ordinary yeast. Our estimates of the iron content of anæmic yeast agree with Elvehjem, and the figures quoted above show that the spectrogram is in agreement, but the spectrogram shows an increase of copper. Two points must, however, be considered. First, that the dose of anæmic yeast given was never more than one-third the amount of yestamin; this would make the actual amount of copper consumed less than in yestamin. Secondly, that the amount of copper present in the specimen of yestamin used in our ' preliminary experiments with yeast '

was the minimum effective dose required as a supplement to 0.5 mgrm. of iron and that the amount of iron present was the minimal daily iron required as an addition to a whole milk diet in the growing rat (Beard and Myers[5]). The assumption, therefore, that the cure of anæmia by anæmic yeast cannot be regarded as due solely to copper and iron is, we submit, abundantly justified.

With regard to marmite and the yeast extract the spectrogram and quantitative estimations are at variance. It is, of course, obvious that different specimens vary considerably in the mineral content, as we have seen yestamin itself does. As far as copper is concerned it is clear that both the extracts and especially the yeast extract are rich in copper when compared with the other preparations, and this is borne out by the quantitative estimations. The spectrogram, however, shows they are both free from iron, whereas quantitatively we have found that they have an amount of iron as great or greater than that in yestamin, and the actual daily amounts of iron available for each rat of which the progress is shown on Graph V were as follows:—

0.16 mgrm. of iron in rats receiving 0.5 grm. marmite.
0.247 mgrm. ,, ,, ,, ,, ,, 1.0 grm. yeast extract.
0.124 mgrm. ,, ,, ,, ,, ,, 0.5 grm. ,, ,,

Whatever is the explanation of the discrepancy between the quantitative method of Hart and Elvehjem and the spectrogram, one thing is quite clear, namely, that the effective cure of the anæmia produced by 0.5 grm. of marmite and 0.5 grm. of yeast extract cannot be due, at any rate entirely, to the presence of iron because, to repeat, it is less than the minimal daily amount of iron (0.25 mgrm.) required as an addition to a milk diet to cure anæmia in the growing rat. The effective possibilities in these two extracts would therefore appear to be amino-acids, copper and vitamin B, and as the copper is ineffective except as a supplement to an adequate supply of iron it would appear that the cure is largely due to amino-acids or vitamin B, or both.

The residue remaining after the special yeast extract had been made is also comparatively rich in copper and according to the spectrogram contains more iron than yestamin, an observation which is borne out by quantitative estimations. The important difference between this preparation and the yeast extract and yestamin itself is probably that it is much poorer in vitamin B, and this may account for the fact that it appeared slightly less efficacious.

The results of ashing the extracts in an attempt to destroy vitamin B complex, and the few experiments we have so far carried out of feeding B_2 to anæmic rats, point to the conclusion that the B complex has some influence on hæmatopoiesis but that unaided it cannot be regarded as an effective curative agent. We have also quoted results which support the suggestion that the B complex is low in anæmic yeast and probably has little to do with its curative effect. The similarity to hæmoglobin of cytochrome which is composed of hæmatin compounds and the unbound

hæmatin of the yeast cell naturally raises the hope that herein might be found an explanation of the action of yeast. This possibility has really been discussed under the heading of iron as actually these hæmatin compounds must be the source of iron in anæmic yeast if that material is free from inorganic iron. Moreover, as we have already stated, Elvehjem has produced definite evidence that hæmatin in the presence of copper promotes a partial cure of anæmia in the rat, but that recovery is neither so rapid nor so complete as when ferric chloride is used as the source of the iron.

There remain for discussion the amino-acids. We regret that we have no direct observations to offer as to their value except the action of marmite. Fontés and Thivolle found that the subcutaneous injection of tryptophane and histidine, or better still, of both these amino-acids, led to an increase in hæmoglobin and red cells in both dogs and rabbits. Drabkin and Miller[4] produce the cure of nutritional anæmia in rats by the addition to milk of pure amino-acids which were copper free and contained only a trace of iron, and that they found the most effective were arginine and glutamic acid. It is interesting to note from the table of amino-acids in yeast given above that glutamic acid and arginine are present in large quantities, and that tryptophane and histidine do not form a large proportion. It will be remembered that Elvehjem repeated some of the experiments carried out by Drabkin and Miller, and was unable to confirm their results when the glutamic acid used was perfectly pure, and at the moment of writing this view of the ineffectiveness of amino-acids is usually accepted.

We have not achieved a complete answer to the question we have set ourselves. At present the partial answers we have been able to give appear like pieces in a jig-saw puzzle, some of which it has been possible to piece together, perhaps inaccurately, but the complete picture has so far eluded us. It is not improbable that all the factors, copper, inorganic iron, organic iron (cytochrome and hæmatin), vitamin B and possibly the amino-acids, are concerned in the cure and that the beneficial results of yeast are due to the summation of all of these. Of these factors it is probable that copper and iron are the most important. It is interesting to note, as will be pointed out in another paper, that yeast is nothing like so efficacious in the treatment of nutritional anæmia of infants as it is in rats, a fact for which we cannot advance any really adequate explanation. It does sometimes produce a slight reticulocytosis in infants and in two instances as an addition to iron produced a cure when iron alone had failed. This result may have been due to the copper of the yeast or, perhaps more likely, to all the factors mentioned above.

Summary.

Yeast has a curative effect on nutritional anæmia of the rat; animals so cured are capable of reproduction and the progeny show nutritional anæmia shortly after birth. Details are given of experiments planned to

determine what are the curative factors in yeast and evidence is brought forward showing that the beneficial effects are due in part to iron (organic and inorganic), copper, vitamin B complex, and possibly to amino-acids.

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Part III.—The anhæmatopoietic anæmias (deficiency diseases of the erythron): nutritional anæmia, and the anæmias of prematurity, scurvy and cœliac disease

BY

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AND

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The widespread prevalence in infancy of hypochromic anæmia, the result of a deficiency of iron in the diet, has been stressed within recent years by Helen Mackay, and in their experimental work on nutritional anæmia in rats Hart, Steenbock, Waddell and Elvehjem have shown that small amounts of copper as supplements to iron are essential for the cure of this condition. The question whether iron alone can produce a cure of experimental nutritional anæmia or if other metals can replace copper as a supplement has been referred to in a preceding paper (Part II). Absence of certain metals from the diet is not the only dietetic deficiency associated with the development of hypochromic anæmia; for instance, the anæmia of scurvy cannot be cured unless adequate amounts of vitamin C are consumed, and the anæmia occasionally found with hypothyroidism or cretinism is said to react specifically to thyroxin. According to Witts[1] iron, copper, vitamin C and thyroxin are necessary for the maturation of normoblast to erythrocyte, and in adults the failure of this change results in the development of a microcytic anæmia. We have been able to demonstrate by means of the hæmatocrit and by Price-Jones curves (Fig. 1) that the nutritional anæmia of infants is also of the microcytic type.

From the recent work of Strauss and Castle[2] the interaction of two factors would appear to be essential for the maturation of megaloblast into normoblast and the consequent prevention of megalocytic anæmia. These factors are:—(a) an intrinsic factor present in normal gastric juice, and (b) an extrinsic factor which they believe to be vitamin B_2. Megalocytic anæmia may also result if there is a failure of absorption or of utilization of the product of the interaction of these two factors.

Deficiency anæmias of the megalocytic type are extremely rare in childhood, but they have been recorded in association with cœliac disease, and even pernicious anæmia itself has been described. The megalocytic anæmia

of cœliac disease, the megalocytic anæmia of the tropics, and that of some cases of sprue, are due to the absence of the extrinsic factor; pernicious anæmia to the absence of the intrinsic factor; and the anæmia associated with dibothriocephalus latus, in all probability, to lack of absorption of the product of the interaction of the two factors.

There are two methods of proving that a disease is due to the absence or inadequate supply of some dietetic factor:—(a) the experimental method of excluding the factor from the diet and thereby inducing the disease; and (b) the therapeutic method, of supplying the missing factor to the individual suffering from the disease and effecting a cure. It is obvious that the therapeutic method is the only one applicable to the child, although in times of warfare or economic stress the experimental method may be forced upon the children of the nation affected.

1. Nutritional anæmia of infants.

For many years some pædiatricians have recognized the existence of a nutritional or alimentary anæmia of infants, but it is only recently that the condition has aroused general interest. The investigations of Helen Mackay have demonstrated that nutritional anæmia of a mild degree is almost universal in both breast-fed and artificially-fed babies; that not infrequently a considerable degree of anæmia develops; that it is most marked in babies who are small at birth, and who, relative to their initial weight, increase in size rapidly; and that it is due to iron deficiency which can be remedied by the addition of a medicinal salt of iron to the dietary, although the beneficial therapeutic effect is diminished by the presence of an infection. In her report to the Medical Research Council Mackay[3] has not only summarized the literature so adequately that it is only necessary to refer to a few of the more important contributions, but she has also given the results of her investigation of the hæmoglobin of some thousands of children, the great majority of whom were regarded by their parents as healthy. We have studied by comparison a small number of cases, but they have all been sick children, and the hæmatological investigations have been more extensive than were necessary or possible in her series.

In the infant, nutritional anæmia resulting from iron deficiency may occur either from deficient storage of iron in intra-uterine life, or from an inadequate supply of iron in the diet, or from a combination of both factors. As far back as 1889 Bunge demonstrated that in certain animals the ashed residue of the fœtus and of its mother's milk contained the same percentage of all minerals with the exception of iron, which was six times more concentrated in the fœtal than in the milk ash. He also found that the amounts of iron per kilogramme of body weight, and of iron in the liver and spleen, were highest at birth and progressively fell, reaching a minimum at the end of the suckling period. Ten years later Hugounenq showed that in the human fœtus two-thirds of the iron present in the body of the full-term infant is laid down during the last three months of pregnancy. The progressive diminution of the iron stored in the liver during the lactation period has been confirmed

by later workers. Copper is stored in like manner to iron: thus, McHargue and his colleagues found that the liver of calves contained more copper than those of older animals, and that the copper content of infantile liver is about 20 mgrm. per cent. of its dried weight. We have been able to demonstrate that the fall in the copper stores of the liver in the infant during lactation is a continuous one similar to that of iron. Observations made by us and reported elsewhere[5] on the amounts of copper and iron present in the carcasses of rats from birth to the end of the weaning period, showed that although the absolute amount of both these elements rose during this time, copper, like iron, when expressed as amounts per kilogramme of body weight, showed a progressive diminution.

As far as essential minerals are concerned, and taking iron as the example, the shortage causing anæmia may therefore occur as follows:—

1. Deficient ante-natal storage.
   (a) Iron deficiency in the mother.
   (b) Deficient transference of iron to fœtus.
   (c) Prematurity of fœtus resulting in insufficient storage.
   (d) Twins, the iron stores obtainable from the mother being insufficient for both children.
2. Deficient post-natal supply.
   (a) Insufficient supply of iron in breast milk, possibly due to iron deficiency in mother's diet.
   (b) Artificial feeding with cows milk which contains less iron than human milk, and gives an iron retention only one-fifth of that in a breast-fed baby (Blauberg).
   (c) Prolongation of milk feeding beyond the normal lactation period.
3. Deficient ante-natal storage and post-natal supply.

The most recent contribution on the question of iron storage is by Gladstone[6]. He finds that the iron contents of the liver and spleen of the infant do not reach their maximum at birth, but at the age of one to ten weeks; that there is no evidence of large or progressive deposits of iron in the liver and spleen during the last four months of intra-uterine life; and that the large amount of iron present in those organs results from the physiological hæmolysis which occurs at birth. Our observations on the livers of premature and new-born infants are not sufficiently numerous to enable us to criticize Gladstone's findings, but H. Ramage, J. H. Sheldon and W. Sheldon[7] have carried out spectrographic examinations of a large series of human livers, and find that copper and iron are the only metals stored as reserve material in the liver during fœtal life and reach their peak at birth ' when figures as high as 0.06 per cent. (dry) for copper and 0.4 per cent. (dry) for iron may be obtained. After birth these figures drop steadily until the stable value is reached at the age of 12 to 18 months.' In a personal communication J. H. Sheldon has informed us that there may be a rise in the iron content of the liver in the early weeks and that this, as Gladstone suggests, is probably

due to iron derived from hæmolysis. Gladstone's arguments do not appear to us to furnish a satisfactory explanation of the frequent occurrence of nutritional anæmia in the premature infant or in twins, whereas those based on the pre-natal storage of iron do afford, as will be obvious later in this paper, a reasonable solution of this problem.

Congenital nutritional anæmia.—Anæmia due to deficient ante-natal storage may be present at or shortly after birth, and forms one variety of congenital anæmia or anæmia of the new born.

Congenital nutritional anæmia differs from nutritional anæmia of later infancy only in the fact that the child may come under observation shortly after birth, or that there is a history of pallor from birth. The subject of anæmia of the new born is discussed at greater length in the paper on the hæmolytic (erythronoclastic) anæmias of the neonatal period (Part IV of this series) because it is probable that in the majority of instances the anæmia is hæmolytic in character. Nevertheless, we maintain that there is a form of anæmia which is present at or shortly after birth, neither hæmolytic in type nor due to any obvious disease, which is a nutritional anæmia curable by supplying the missing dietetic factors. Such cases have been described by one of us[8], and their counterpart in the rat has been discussed in the preceding paper (Part II). Before the identification of anæmia in a new-born child as a congenital nutritional anæmia can be accepted, in addition to the history of anæmia since birth or the presence of pallor in the new-born child, it is essential to exclude the occurrence of an abnormal hæmolysis. This may be done, first, by the absence of icterus and of a sudden dramatic increase in anæmia shortly after birth; secondly, by the presence of a blood count showing a typical hypochromic anæmia and without evidence of a marrow response; and thirdly, by the occurrence of a reticulocytosis after treatment with the missing factor or factors. If pathological hæmolysis has occurred the blood picture is quite different, the anæmia is of the hyperchromic type, and unless the marrow is completely paralysed there is a marked reticulocytic response before or without treatment by any of the hæmatopoietic factors such as iron which produce a cure in nutritional anæmia, proving that the infant has no shortage of these factors and can at once start to turn out new cells to replace those which have been destroyed.

The nutritional anæmia of later infancy and early childhood.—This differs in no way from the congenital form, or from the nutritional anæmias of early infancy, except that it is not noticed until the seventh month of life or later. These children have usually been fed on cows' milk or artificial food, but in the instances in which anæmia has occurred in the breast-fed child it has been due to the continuance of breast feeding beyond the normal weaning period, or to the fact that the child has suffered from some infection.

The nutritional anæmias of earlier infancy.—This constitutes an intermediate group between the two foregoing forms. In it the pallor is first noted at about the fourth month and it is probable that there is a combined pre-natal and post-natal deficiency. The incidence of this variety of anæmia is higher in twins than in single pregnancies; thus, out of 30 of our most severe nutritional anæmias, all in this group, 8 were twins.

Ætiology of nutritional anæmia.—The diets taken by the mothers of infants suffering from congenital nutritional anæmia are not infrequently very inadequate and the mothers themselves may exhibit hypochromic anæmia. Their obstetric history may reveal the occurrence of still-births or of weakly babies, and we are of opinion that congenital nutritional anæmia can be prevented by an adequate dietary for the mother, if she is otherwise healthy. This view is directly contrary to that held by Baar and Stransky[9] who believe that the anæmia of the mother does not produce anæmia in the new-born child. The maternal diet is also an important ætiological factor in the nutritional anæmia of early infancy. Following on Helen Mackay's findings, and using the administration of iron as a therapeutic test that the anæmia is nutritional in origin, Neale and Hawksley have been able to collect a series of cases in which a nutritional anæmia has occurred in mother and child (in some instances twins) in all possible combinations and permutations which can be diagrammatically represented as follows:—

	1	2	3	4	5	6
Mother	N	N	N	A	A	A
Infant	N	A	A	N	A	A
Twin	N	N	A	N	N	A

N=Normal. A=Anæmic.

The clinical details and discussion of these cases are contained in a subsequent paper (Part VI), and it suffices to point out here that some mothers have the power to retain their iron to the detriment of the fœtus, a state of affairs entirely different from that which obtains with calcium, although in other ways the metabolism of these two elements in the body is very similar.

Clinical characters of nutritional anæmia.—The children are usually fairly well nourished and of normal size for their age, and their appearance is striking only because of their pallor. This varies from a slight to an extreme degree of whiteness according to the intensity of the anæmia. There is an absence of any icteric tinge of skin or conjunctivæ and the mucous membranes are pale. Before the existence of an anæmia can be recognized by inspection the hæmoglobin must have fallen to somewhere in the neighbourhood of 60 per cent., and even at that figure if the child be near a fire or begins to cry the colour will improve so much that the anæmia is not obvious. The spleen is often palpable, but the lymph glands are of normal size. Evidence of infection in the form of bronchitis or otorrhœa is, as emphasized by Mackay, frequently present. No other findings are constant, but we have noticed that the proportion of nutritionally anæmic infants who show mental backwardness is greater than that found in normal children. This fact is perhaps related indirectly as cause and effect, because the difficulty in getting such children to take a mixed diet leads to prolonged milk feeding. Some of the children suffering from nutritional anæmia of later infancy are also the subjects of rickets, but there is no evidence that the anæmia and rickets have any relationship, except that a diet which is deficient in one factor is also

liable to be deficient in other factors. In these instances cure of the anæmia and of rickets can be effected by appropriate treatment, either synchronously or consecutively.

The hæmatological picture varies, but it is always of the hypochromic type, the colour index in a case of moderate severity being about 0.6. The less severe anæmias show a fall in hæmoglobin with little if any diminution in the red cell count, whereas when the anæmia is more severe the hæmoglobin values are still lower, and the fall in the red cells is proportionately rather smaller. Reticulocytes are either absent from the blood or at most reach a figure of 0.4 per cent. of the red cells. Isolated readings of reticulocytosis as high as 1.5 per cent. have been met, but these are always very transient. Price-Jones curves (Fig. 1) show that the anæmia is of the microcytic variety. In the more severe cases a few normoblasts are found in the peripheral blood. The white cells do not show any constant qualitative or quantitative changes and reactions to infections are often present. The number of platelets is normal or slightly reduced, and the fragility of the red cells is normal.

The blood counts in a few typical cases of nutritional anæmia of infants, demonstrating the varying degrees of the severity of the anæmia, are shown in the following lists:—

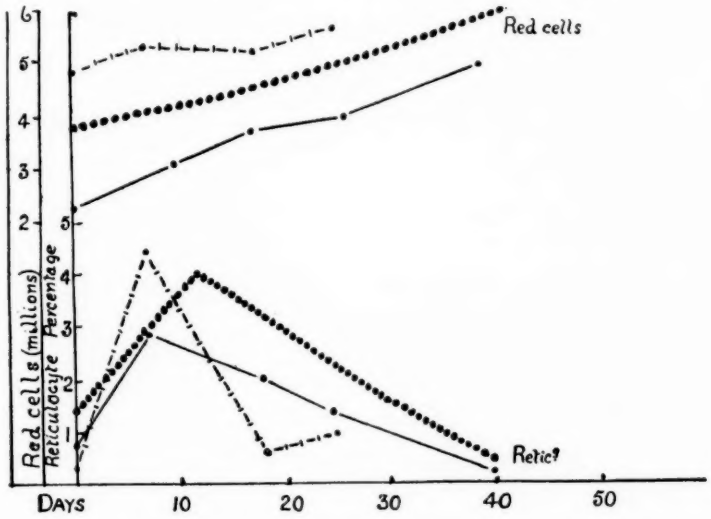
Red cells	4,520,000	4,300,000	4,244,000	3,302,000	2,120,000
Hæmoglobin per cent.	55	55	48	28	16
Colour index	·6	·64	·57	·42	·4
Leucocytes	17,500	13,000	10,300	5,800	8,400
Reticulocytes per cent. of red cells	0·5	0	0·2	0	0·8
Platelets	190,000	220,000	444,000	plentiful	160,000
Neutrophil polymorph.	37	38	54		20·5
,, metamyelocytes	1		0·5		2
,, myelocytes ...					1·5
Eosinophils per cent.	5	1	0		0·5
Basophils per cent. ...	nil	nil	1		1 0
Lymphocytes per cent.	53	52	39		61·5
Plasma cells per cent.		0·5			
Türck cells per cent.					0·5
Monocytes per cent. ...	4	8·5	5·5		12·5
Normoblasts per cent. of leucocytes	0	0	0	0	5·5
Macro-normoblasts per cent. of leucocytes ...	0	0	0	0	1
Megaloblasts per cent. of leucocytes	0	0	0	0	0

Iron therapy.—The method used in determining which factor is deficient in the diet has been to give the child a medicinal preparation of iron. Usually this has produced a cure, but sometimes only a partial recovery occurred, and then another dietetic factor has been added and further effects noted. The diet itself has not been changed and no other treatment given unless this

has been essential because of untoward symptoms. We believe that the experiments have been thus controlled as far as is possible in a clinical investigation.

The preparation most often used has been reduced iron, which has been given in doses of one half to one grain three times a day. The results of adding iron to the diet of an infant suffering from nutritional anæmia due to lack of iron are as follows:—a reticulocytosis; a fairly rapid rise of erythrocytes to normal or slightly above normal, counts of over six million per c.mm. being regarded as excessive; a more gradual return of hæmoglobin to normal; and finally a reduction in the number of red cells to normal directly after the final stages of the hæmoglobin rise, a phenomenon first recorded by Hagen. Accompanying these changes there is marked improvement in the physical condition; colour returns to the skin and mucous membranes, the child becomes more active although the weight does not necessarily increase, and catarrhal infections, which have previously been resistant to treatment, begin to improve. If splenomegaly was present before the commencement of treatment it may persist until some considerable time after cure has been obtained, although it is quite probable that in many cases infection may be an important factor in causing the splenic enlargement.

Some of these points are demonstrated on Graphs I, II and III. Graph I shows the effect of iron on the red cells and the degree of reticulocytosis

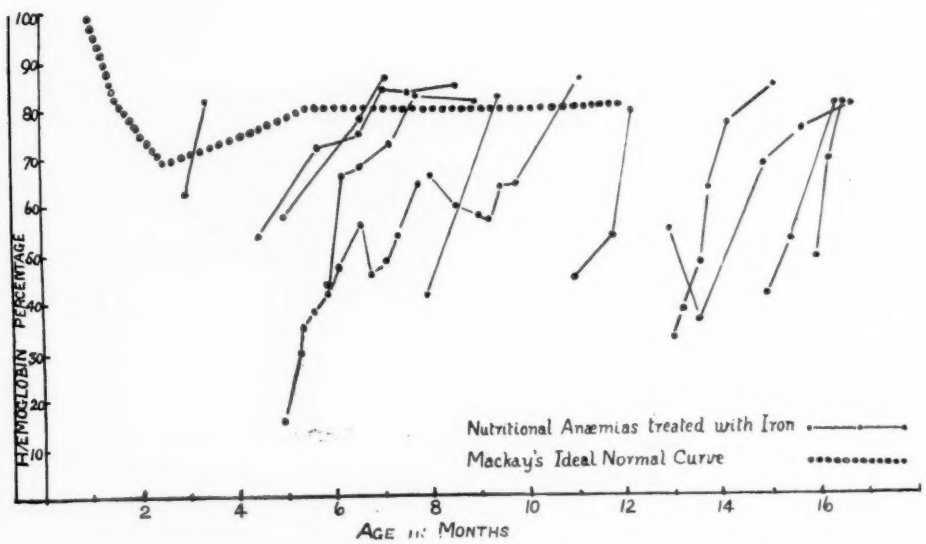


GRAPH I.—Effect of iron on reticulocytes and red cells in anæmia due to iron deficiency.

produced in three severe cases of anæmia; and Graph II illustrates the effect of iron on the hæmoglobin of infants of differing ages, showing that in each case the hæmoglobin has risen to the normal height for the age. The variations in the rates of recovery in these cases are remarkable and rather difficult to understand. There are at least four possible explanations: first,

the presence of an intercurrent infection; secondly, differing degrees of regenerative power owing to the abeyance of marrow function; thirdly, varying degrees of iron shortage; fourthly, the preparation of iron used. Reimann and Fritsch[10] have recently shown that reduced iron is only effective by virtue of the formation of ferrous chloride by the action of the hydrochloric acid of the gastric juice. Hypo-acidity of the gastric juice is not infrequently found in sick and weakly infants and it is possible that thereby the amount of iron absorbed may vary in different individuals and even in the same infant at different times. The failure of the child to respond to reduced iron when an infection is present may also in part be explained by this mechanism since in infections the gastric juice shows hypo- or even an-acidity.

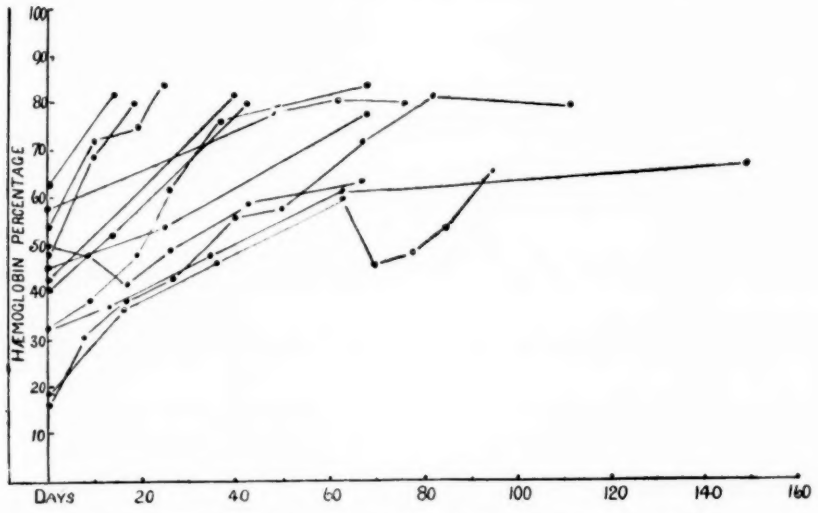
Copper.—The experience of Hart and his colleagues that, whereas impure inorganic salts of iron cured nutritional anæmia, the pure salts failed to do so unless supplemented by small amounts of copper, have already been



GRAPH II.—Effect of iron on hæmoglobin in infants of various ages.

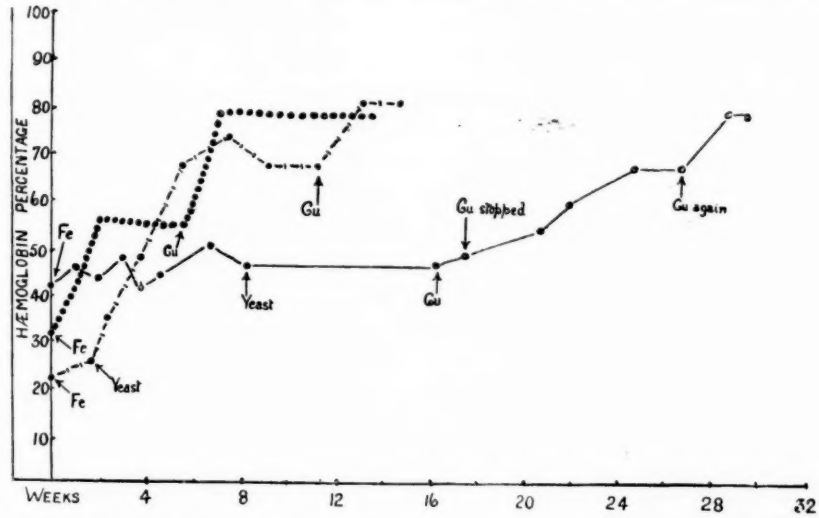
applied by clinicians in the treatment of anæmia. The most carefully controlled experiments are those by Josephs,[11] who compared a series of cases of anæmic infants treated with iron with a series in which both copper and iron were present. He found that in the latter series in which copper was used as a supplement a more rapid rise in hæmoglobin occurred. In a few of our cases, where the hæmoglobin failed to rise to normal with iron, the effect of adding other substances was tried. In one case with iron therapy the hæmoglobin rose, but did not reach normal, and actually a continuance of the treatment led to the rising curve of hæmoglobin becoming flattened out and eventually being replaced by a fall. In another case treatment with iron and yeast over a period of several months failed to produce any effect. All these cases responded rapidly and the hæmoglobin rose to normal

(Graph IV) when copper was added. The form in which copper was administered was that advised by Josephs, namely 1 c.cm. of a 0·5 per cent. solution of copper sulphate per kgrm. body weight given daily in milk. Although the addition of copper resulted in the hæmoglobin becoming



GRAPH III.—Effect of iron administration on hæmoglobin.

normal, there was no effect on reticulocytosis nor on the red cell count. This was originally observed by Josephs and indicates that, although copper is necessary for hæmoglobin synthesis, it has no effect on the manufacture of



GRAPH IV.—Effect of copper as a supplement to iron, or iron and yeast.

red cells. The number of cases in which we have used copper as a supplement to iron has been small, but the experiments were carefully controlled as described above and the results can, we submit, be accepted as examples of the action of copper.

Relative importance of iron and copper in nutritional anæmia.—The mechanism whereby copper produces its effect has been a subject of considerable speculation and experiment. Several workers have come to the conclusion that copper mobilises the iron in the liver, and Elvehjem and Sherman[12] have shown that in nutritional anæmia of the rat in the absence of copper, inorganic iron is readily assimilated and stored in the liver and spleen. The iron so stored cannot be used for hæmoglobin formation until copper is supplied, when the greater part of the iron in the liver is removed and built into hæmoglobin. Copper is not necessary for iron assimilation, but it is necessary for the synthesis of hæmoglobin. Applying these experimental investigations to the three cases we have described it would appear that owing to deficiency in the copper supply these infants had depleted their available copper storage and were unable to turn their absorbed iron into hæmoglobin until further copper was supplied.

In attempting to assess the relative values of iron and copper in treatment such factors must be borne in mind as the variation of the amount of iron in milk from different countries, in different samples from the same cow or from different cows; its increase from storage in iron vessels, and the possible increase produced by the method of drying milk, which have been discussed in the preceding paper (Part II). Again it is important to remember that according to J. H. Sheldon[7] at least 80 per cent. of medicinal preparations of iron contain copper as an impurity, and that the amounts of copper present in the various preparations are not constant, varying not only with the particular preparation used, but also in individual specimens of the same preparation. The difficulties in determining the real importance of copper in the treatment of nutritional anæmia in infants are obvious, nevertheless it is advisable that this should be attempted. From the evidence we have put forward and from Joseph's work it is obvious that in some cases it is necessary to give copper completely to cure the anæmia. The question we have to answer, if possible, is whether all children suffering from nutritional anæmia require the administration of copper to ensure recovery; or to put the question in another way:—can the administration of a pure salt of iron cure nutritional anæmia in the infant?

It will be remembered that whereas the views of Hart and his colleagues that pure iron will not cure nutritional anæmia in the rat are generally accepted, Beard and Myers contend that a cure can be obtained with iron only. Iron is stated by Heubner[13] and by Starkenstein[14] to be effective in anæmia only if it is absorbed in the divalent condition, i.e., as a ferrous salt; and the work of Reimann and Fritsch[10] showing that reduced iron is only effective when converted into ferrous chloride in the stomach has already been quoted. In view of these statements we were anxious to try the effect of giving pure ferrous salts to children in nutritional anæmia, but we have been unable to purchase ferrous chloride which is guaranteed free from copper.

Ferrous sulphate has therefore been used, the particular preparation being ferrous sulphate (A.R.) made by British Drug House, Ltd.

A difficulty in using ferrous salts for medicinal purposes is that their solutions readily take up oxygen and thus become converted to the ferric form.

In Germany this difficulty has been overcome by giving them in tablet form, but such a form of medicament is unsuitable for infants. We have, however, found that oxidation can be prevented by using syrup as a vehicle and this has been prepared by dissolving pure glucose (as used for intravenous injection) in water to form a thin syrup. The scale preparations of which iron and ammonium citrate has been used by Helen Mackay are undoubtedly efficacious in nutritional anæmia, and it is probable that in the stomach they are slowly broken down and ferrous salts formed. The iron content of various iron preparations have been set forth in a table by Clark[15], which shows that 1 grn. of reduced iron (B.P.) contains 54 mgrm., 1 grn. of ferrous sulphate (B.P.), 12 mgrm., and 1 grn. of iron and ammonium citrate (B.P.), 13·5 mgrm. of iron respectively. Assuming that 3 grn. of reduced iron daily is an average dose for an infant suffering from nutritional anæmia, approximate equivalent amounts of iron would be supplied by 13½ grn. of ferrous sulphate, and 12 grn. of iron and ammonium citrate. We have therefore given 12 grn. of pure ferrous sulphate daily divided into three doses, an amount which in iron value is slightly greater than the iron value of the maximum dose (9 grn.) of iron and ammonium citrate used so successfully by Mackay. If the ferrous salt is more efficacious than the scale preparation better results should be obtained by using this drug, and this has in fact proved to be the case. By a happy chance we had the opportunity of showing to Dr. Helen Mackay some infants under treatment with ferrous sulphate, and she informed us that the response we had obtained was quicker than anything she had seen with iron and ammonium citrate. The following case may be quoted to illustrate these points:—

Case 1.—H. M., male, aged 4 months, the survivor of twins, had been fed on cows' milk and water and was admitted to hospital (19.10.32) for anæmia and loose stools. On physical examination with the exception of anæmia nothing abnormal was discovered. An examination of the blood showed that the hæmoglobin was 50 per cent., the red blood cells 4,231,000 per c.mm. and the colour index 0·59, and there was a reticulocytosis of 1·4 per cent.

In the paper on nutritional anæmia in the rat, mention has been made of the extraordinarily rapid increase in hæmoglobin which took place when anæmic rats were given ferrofax (powder). Ferrofax is prepared by British Colloids, Ltd., and contains iron, copper, manganese, and yeast. We therefore tried the effect of giving ferrofax (liquid) to this child; this was started on October 24th and four days later a reticulocyte response of 3·2 per cent occurred. The ultimate result was very disappointing, for although there was an increase in red cells by one million per c.mm., the hæmoglobin only rose 4 per cent. as a result of 17 days treatment. On the 13th November, therefore, pure ferrous sulphate was started in daily amounts of 12 grn., and the result was extraordinarily good, for in an exactly similar period of time the hæmoglobin rose by 21 per cent.

The results of this experiment were communicated to Mr. John F. Ward, Chief Chemist to British Colloids, Ltd., who, in giving us the mode of preparation of the two forms of ferrofax, pointed out that ferrofax powder is largely ferrous iron, whereas ferrofax liquid is ferric iron. This not only explains why the result in the child differed so much from that obtained in

rats, but taken in conjunction with the improvement which occurred after the administration of ferrous sulphate, also shows that iron in the ferrous form is greatly superior to iron in the ferric form. These two varieties of ferrofax are manufactured as follows:—

Ferrofax (powder). Freshly precipitated ferrous carbonate is mixed with a mixture of glucose and sugar, and dried. By the use of glucose the iron is largely preserved in the ferrous state. The dried powder is mixed with an equal weight of yeast powder and a definite proportion of copper hydroxide and manganese hydroxide added; when mixed with water the powder forms a fine colloidal suspension.

Ferrofax (liquid). This is made by precipitating ferric hydroxide and carefully washing until free from salts. It is then mixed with sugar and evaporated to dryness. The powder is then dissolved in water and a true ferric hydroxide colloid is formed. The necessary amount of manganese is added to the final product.

In view of the result of administering ferrofax liquid to this child, British Colloids, Ltd., have now prepared a new ferrofax liquid. In the new preparation ferrous hydroxide is formed in the presence of glucose solution which on account of its reducing action keeps the iron in the ferrous state.

Although the amount of copper in ferrous sulphate (A.R.) is scarcely sufficient to estimate (the makers say less than 1 mgrm. per cent.), we have found that the ' pure ' glucose prepared for intravenous use unfortunately does contain a definite amount of copper, namely 0·3 mgrm. per cent. The amount of glucose consumed daily by this child was 11·25 grn. and less than 1 grn. of ferrous sulphate, which means an intake of about 0·031 mgrm. (0·03—0·001) of copper per day. According to Beard and Myers the amount of copper required as an effective supplement to a daily dose of 0·5 mgrm. of iron in rats suffering from nutritional anæmia and fed on milk only is 0·025 mgrm. Accepting that statement, a daily supply of 0·031 mgrm. of copper would be manifestly insufficient to activate the amount of iron given to this child which was 144 mgrm. We, however, recognize that a mathematical argument of this type will not compare in value with clinical results obtained by completely eliminating the copper intake, other than that contained in the milk diet, and we are now endeavouring to accomplish this. There is in this particular instance an adequate reply to the suggestion that copper took no part in the cure because the child had been given copper in ferrofax (liquid) which doubtless had been stored in its body, and was therefore available for hæmoglobin synthesis when a sufficient amount of iron was forthcoming. Such a reply is, however, not applicable to the next case:—

Case 2.—P. F., male, aged 8½ months, a premature twin, fed from birth on a roller process dried milk, was admitted to hospital for nutritional anæmia on December 14th, 1932. Except for the anæmia and an attack of diarrhœa in association with an influenzal infection, which occurred during the fourth week of his stay in hospital, there was nothing of importance in his physical condition. At the outset his red blood count was approximately normal but the hæmoglobin was 57 per cent. He was in fact a mild case of nutritional anæmia and a Price-Jones curve (Fig. 1) of his blood on the day after admission showed a very definite degree of microcytosis and a moderate degree of anisocytosis. On December 17th he was put on pure ferrous sulphate, 12 grn. per day made up with glucose as before, and 14 days later his hæmoglobin had increased to 70 per cent., and in spite of the influenzal infection his hæmoglobin had reached 82 per cent. on January 13th, 1933. Another Price-Jones

curve done on January 21st, although still showing a degree of microcytosis and even an increase in anisocytosis, had moved well to the right into the normal area. A final Price-Jones curve (March 8th, 1933) showed very little anisocytosis and is otherwise a normal curve. This series of curves demonstrates very well how the size and shape of the red cells alter under treatment.

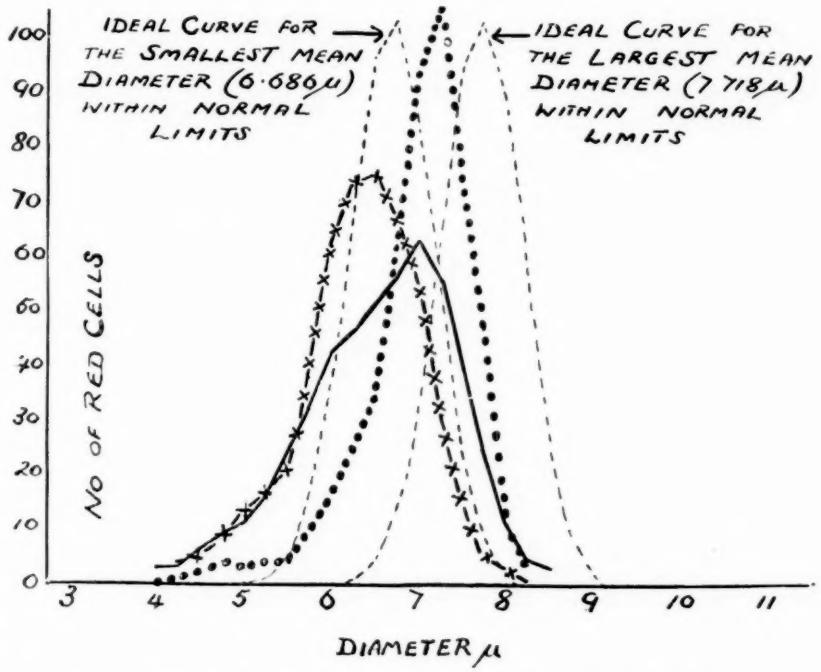


FIG. 1.—Case 2. Price-Jones curves showing effect of treatment.

×—× 15.12.32. before treatment with iron: mean diameter 6·348 μ.

——— 21.1.33. after five weeks' treatment: mean diameter 6·559 μ.

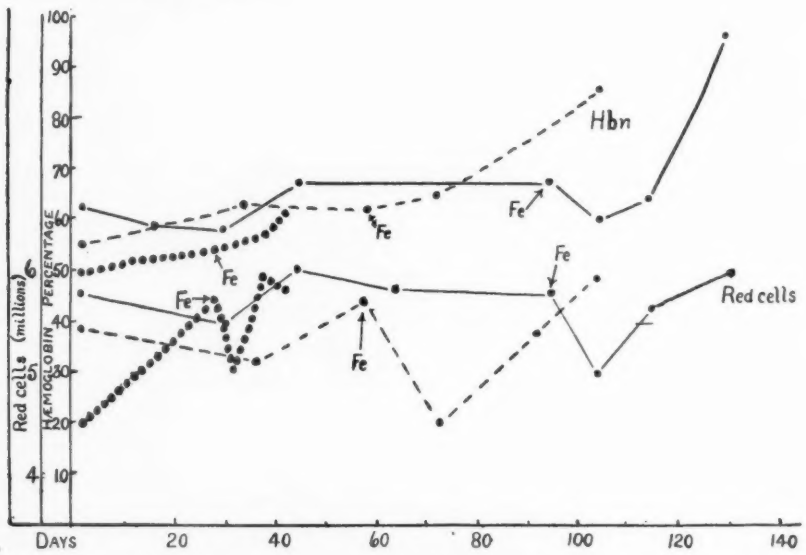
. . . . . 8.3.33. curve within normal limits: mean diameter 7·001 μ.

It is difficult to understand the rationale of cure in this case if copper in the amounts indicated is as essential for the cure of nutritional anæmia in the infant as it is in the rat. The suggestion that the child had sufficient stored copper for use when adequate iron supplies were forthcoming is a possible explanation, although the acceptance of such a solution is not easy because the only source from which copper could be obtained was from the mother in utero. It is, of course, also a possibility that this child had never had sufficient ante-natal iron storage nor a sufficient post-natal supply of iron to make use of all its stored copper; a possibility which seems unlikely because it is usually assumed that the ante-natal iron and copper storage proceed along parallel lines. In this particular instance it is probably quite true that ante-natal iron storage was poor because the child was both premature (7 months) and a twin.

At the time of writing this is the only case in which we have been able to show the effects of a pure ferrous salt where there was no other possible

source of copper than the amount supplied in the glucose and ante-natal storage. We have had, however, other examples which further demonstrate the value of ferrous sulphate and as will be described later remarkable improvement in the general condition of the child and rapid cure of the hypochromic anæmia of cœliac disease have occurred. We have no hesitation in saying that in our hands ferrous sulphate has proved by far the most effective source of iron in the iron-deficiency anæmias. The results obtained with pure ferrous sulphate do not, of course, vitiate the experiments detailed in the section on copper which show that in some cases at any rate the cure of nutritional anæmia of infants will not take place unless an adequate supply of copper is given to the child.

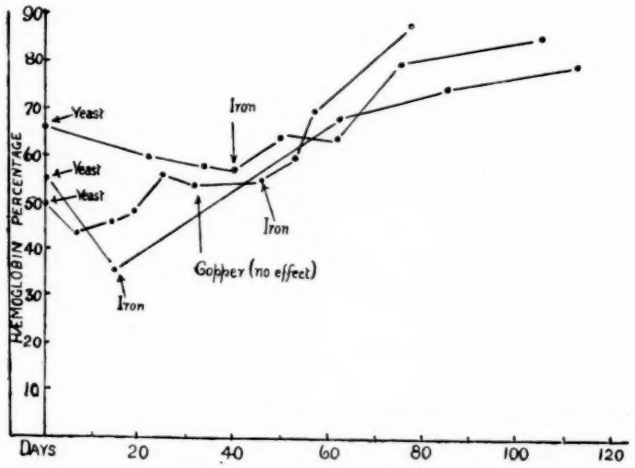
Yeast therapy. — In view of the results of our experiments on rats (Part II) in which nutritional anæmia was cured by adding dried yeast to a milk diet, we investigated the effect of giving yeast to infants suffering from nutritional anæmia. The yeast was given in four different ways: (1) as the sole treatment; (2) given alone at first and later supplemented by iron; (3) as a supplement to iron; (4) in combination with iron from the commencement of treatment.



GRAPH V.—Effect of iron after employment of yeast (3 cases).

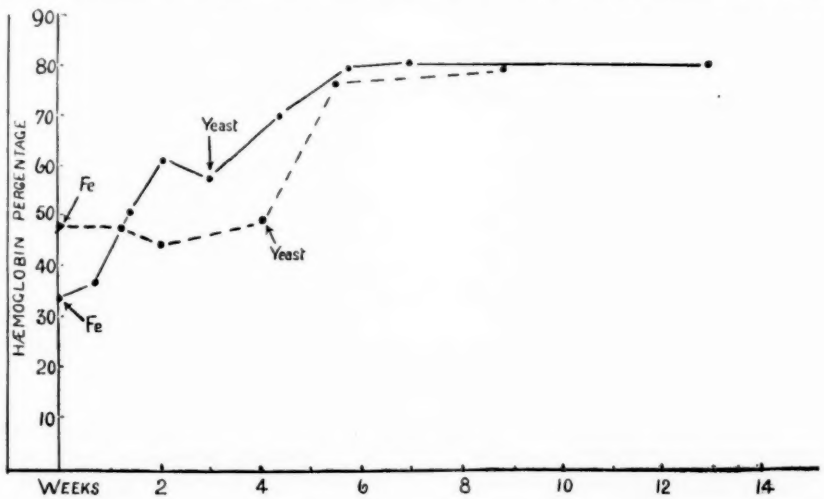
The preparation of dried yeast used was yestamin and this was given three times a day in doses rising from ½ to 4 drm., and as in other forms of treatment the diet, on which the anæmia had either developed or failed to improve, was continued unchanged. The results obtained varied considerably and in only one instance was there complete recovery. In three cases a small reticulocyte 'kick' was obtained on the third or fourth day, and although there was a slight rise in hæmoglobin of about 10 per cent. complete cure was obtained only when the yeast was supplemented by iron; in each instance the addition of iron was followed at first by a fall in erythrocytes from half to one million per c.mm. (Graph V). These infants, however, had

an approximately normal red blood cell count when the yestamin was started and this phenomenon may be that as described by Hagen to which reference has been made above. In three other cases yestamin did not produce any effect, not even a reticulocyte 'kick,' but in each case a normal reticulocytosis and a return to a normal hæmoglobin was produced by the addition of iron (Graph VI). Finally, in two instances in which iron had failed to



GRAPH VI.—Effect of iron after yeast has failed.

produce complete recovery that result was obtained by the addition of yestamin (Graph VII). In these two cases copper was not administered, so that it is possible to argue that the cure was due to the presence of copper in

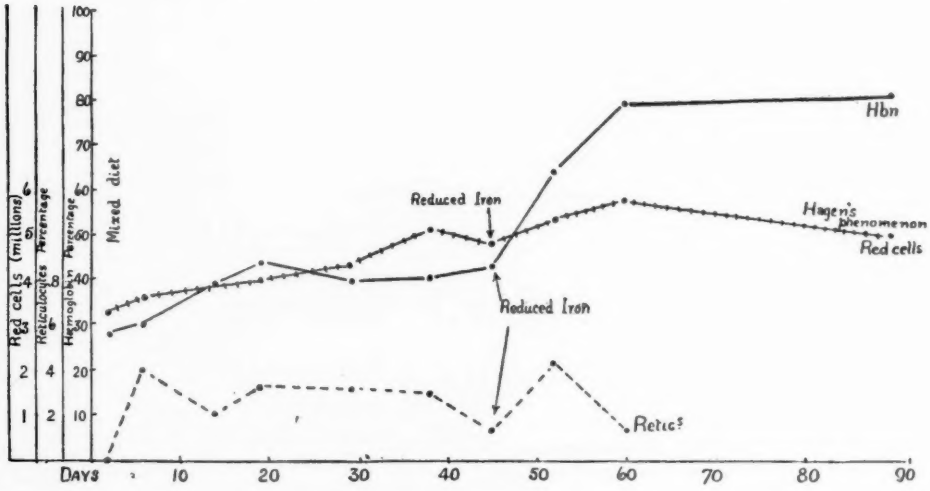


GRAPH VII.—Effect of yeast supplementing iron.

yestamin. We have not arrived at any definite conclusion as to whether iron and yeast given in combination produce a more rapid cure of nutritional anæmia than iron alone; the children of the former group certainly seem to

show greater general improvement but this may well be due to beneficial factors in the yeast which have no direct effect on the hæmatopoiesis. It is obvious, however, that the yeast is much less efficacious in the treatment of nutritional anæmia in the child than in the rat, a state of affairs for which we can advance no adequate explanation. The factor or factors in yeast which have a beneficial effect in hæmatopoiesis have been discussed in the preceding paper.

Natural cure of nutritional anæmia.—When the normal child reaches the end of the lactation period and begins to take a mixed dietary, and in consequence an increased amount of minerals (iron and copper), the hæmoglobin begins to rise and rises steadily until at about the age of eighteen months the adult level is attained. It is quite obvious that many mild cases of nutritional anæmia in this way achieve a spontaneous recovery since, when a purely milk diet is changed to a mixed dietary in nutritional anæmia, a reticulocytosis and gradual improvement in hæmoglobin value occurs. This improvement can be accelerated in any stage by adding iron, particularly as after a time unless iron is given the hæmoglobin curve is liable to become flattened out and only to approach very slowly to the normal. These points are illustrated in a Graph VIII which shows the effect on a child aged 17



GRAPH VIII.—Effect of mixed diet on nutritional anæmia: its reinforcement with iron.

months of changing from a diet which had previously been only milk to a mixed dietary. In this particular case the increase in iron in the diet was from 3·6 to 25·64 mgrm. per diem. The effect of giving extra iron, as reduced iron, is also seen in the graph. It is interesting to note that the most recent work indicates that the iron requirements for growth are 0·2 mgrm. per kgrm. body weight daily, and that the percentage of iron absorbed is increased by increasing the iron intake.

2. Anæmia of prematurity.

In an infant born before term, the number of red cells and the proportion of nucleated cells at birth are higher than in the full term child, and increase in direct proportion to the degree of prematurity. Thus in an eight months fœtus the red cells number about eight millions per c.mm. The changes which take place after birth are essentially similar to those which occur in the full-term new-born child, differing only in that they are more pronounced. The fall in the red cells and hæmoglobin is greater, more rapid and more prolonged, and is likely to be more marked the smaller the infant and the greater the degree of prematurity. From the first week or so, or even up to the fourth or fifth week, the premature behaves like a full-term infant, but after that time shows a much more marked drop in hæmoglobin and a continuous fall in red cells which reach a minimum from the eighth to the twelfth week. At this stage the hæmoglobin is about 45 to 55 per cent. and the red cells $2\frac{1}{2}$ to 3 millions. Subsequently the hæmoglobin begins to rise again in a similar manner to the earlier rebound that occurs in the full-term infant, until at the sixth to eighth month the percentage has reached that of the normal child. The red blood cells also begin to increase in number at the same time and reach the level of the normal rather earlier than the hæmoglobin.

Three main hypotheses have been put forward to explain this so-called physiological anæmia of the new-born premature child.

(1) That it is the result of deficient ante-natal storage of iron, and perhaps of copper, in the liver and spleen, since, as already stated, Hugounenq has shown that in the human fœtus two-thirds of the iron present in the full-term child is laid down during the last three months of intra-uterine life. (2) Josephs[16] maintains that, as in the normal child, the fall in red cells and hæmoglobin is due to the adjustment of blood formation and destruction at a lower level than at birth, but that this failure of the hæmopoietic tissue is more profound and prolonged in the premature child. (3) van Creveld and Heybroek[17] stress the importance of blood destruction rather than a failure of formation and regard many of these anæmias as being of the hæmolytic type and due to an increased severity and prolongation of the hæmolysis which normally occurs at birth. The hæmolysis which normally occurs at birth is due to alterations in oxygen tension. The anoxæmia of the fœtus, which results from its living in a low oxygen tension environment, requires a larger number of red cells and high hæmoglobin; whereas, after birth, the child lives in a high oxygen tension environment and destruction of redundant red cells occurs. The premature infant's need for oxygen is much less than that of the full-term child, and therefore in the premature the destruction of red cells will be greater and last for a longer period. As proof of their view of this increased hæmolysis they point out that whilst the bilirubin in the blood (icteric index) in the full-term child returns to normal at the end of the second week, in the premature child it takes much longer, and until it has reached normal the red cells continue to decrease. In this connection it is interesting to remember that icterus neonatorum is more frequent, more severe and more prolonged in premature than in full-term babies.

Many observations have been made which show that the administration of iron does not prevent the anæmia of prematurity, and it has therefore been suggested that the ante-natal shortage of iron is of no importance in these anæmias. In our opinion this is quite a wrong conception for, on the one

hand, Josephs has pointed out that the initial fall in hæmoglobin in the normal child cannot be prevented by the administration of iron; on the other hand, once the fall of hæmoglobin in the premature infant has reached its lowest point, iron administered in adequate amounts will cure the resulting anæmia. In other words, the storage of iron in the depots of the fœtus, be it great or small, has nothing to do with the initial fall of hæmoglobin, either in the premature or in the full-term infant. The mechanism of the fall in the two groups of children is the same, whether it is, as Josephs says, largely a matter of lessened blood formation, or, as van Creveld and Heybroek maintain, of increased blood destruction resulting from an increased oxygen stimulation, or, as is perhaps most likely, a combination of the two. The question of the adequacy of ante-natal iron storage, and incidentally also of the post-natal supply, only arises when the hæmoglobin has reached its lowest value. In the full-term child this supply may be sufficient to ensure that the child never suffers from nutritional anæmia, but in the premature child with its lack of iron stores, the occurrence of nutritional anæmia is probable, and also it is probable that this will occur early (nutritional anæmia of early infancy). The anæmia of prematurity must be distinguished from nutritional anæmia of the new born, because the latter occurs at an earlier period, shows no evidence of abnormal hæmolysis and is amenable to iron therapy much sooner. It is, of course, possible that anæmia of the new born may occur in a premature infant, and that if the baby did not come under observation until it was two or three months old the anæmia might then be a combination of anæmia of the new born and anæmia of prematurity.

Clinically the appearance of babies suffering from anæmia of prematurity does not differ from those suffering from nutritional anæmia, with the exceptions that (a) splenomegaly and lymphatic hyperplasia are perhaps more common when the anæmia has fully developed, and (b) in the earlier stages the anæmia is of the hyperchromic type. After the anæmia has become fully developed the blood picture changes to that of a nutritional anæmia, but treatment is usually required for a longer period to produce a complete cure. In a previous paper[5], under the heading of 'constitutional nutritional anæmia,' one of us has referred to a group of cases in which anæmia occurred in early infancy and was resistant to treatment. In the light of further experience we should now regard most of these cases as examples of the anæmia of prematurity or of nutritional anæmia of the new-born, and we have found that with improved treatment the number of cases which resist treatment, except during the progress of an infection, becomes smaller and smaller.

The possibility of preventing the development of anæmia of prematurity has been investigated. Abt and Nagel[18] found that if iron and ammonium citrate were administered with the dried liver fraction suitable for a secondary anæmia, made according to the formula of Whipple and Robscheit-Robins, from the beginning of the second week of life, a less severe grade of anæmia developed; whereas the liver fraction alone or the administration of dessicated pig's stomach had no prophylactic value. Van Creveld and

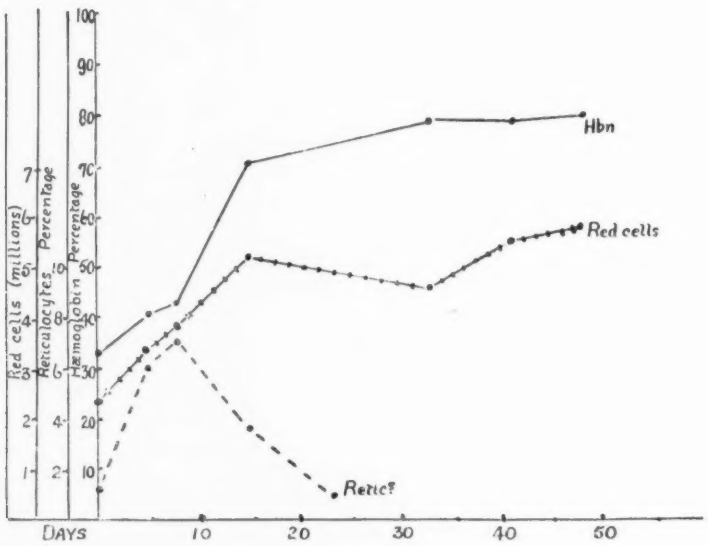
Heybroek found that a combination of liver and vitamin D, or of reduced iron and vitamin D, also prevented the development of severe grades of anæmia. Finally, Blackfan, Baty and Diamond[19] state that the development of this anæmia has been prevented by repeated blood transfusions. Such results encourage the belief that some adequate means of prevention will eventually be found. The beneficial effect of the administration of serum in checking the hæmolysis of icterus gravis neonatorum suggests that such a form of treatment might prevent the development of anæmia of prematurity, and possibly this is the explanation, at least in part, of the good results obtained by repeated blood transfusions. It may be recalled that Hampson[20] has put forward the view that serum supplies an anti-hæmolytic factor which is lacking in icterus gravis, and it is possible that the premature child also is deficient in this factor or in something which stimulates its formation.

3. Anæmia of scurvy.

There is some difference of opinion concerning the frequency of anæmia in scurvy. It is well known that pallor is a characteristic, common, and early symptom of the disease, but whereas A. F. Hess[21] regards this as due in a large measure to anæmia, Rohmer and Bindschedler[22] say that it is not always the expression of real anæmia but only of circulatory changes in the skin. The latter writers point out that anæmia is not an essential symptom, and that a certain number of cases of scurvy without anæmia are recorded in the literature, and quote Baar as having found it in only one-third of his cases. In a series of fifteen cases of scurvy they observed seven instances of anæmia, and are of the opinion that its appearance is connected with certain super-added conditions which do not form a part of true scurvy. The only suggestions they make as to the reason why anæmia occurs in some cases and not in others is that it may depend on the existence of more than one factor in vitamin C and of ' constitutional differences ' in different children.

The anæmia of scurvy which may reach a profound degree is of the hypochromic type and the hæmatological picture does not differ from those other forms of deficiency anæmia to which reference has already been made. It is not possible to say to what extent this anæmia is due to the loss of blood from scorbutic hæmorrhages, although the picture presented is quite different from that which arises from sudden loss of blood. In the latter an attempt is at once made to compensate for the anæmia by regenerative efforts on the part of the bone marrow with the consequent occurrence of reticulocytosis, whereas in scorbutic anæmia this does not happen until after adequate quantities of vitamin C have been taken. It therefore appears that

hæmatopoiesis does not occur in the absence of vitamin C. Graph IX illustrates very clearly the effects on hæmoglobin, red cells and reticulocytes of administering orange juice to a child who became very anæmic during a severe attack of scurvy. No other medication was given and the child was kept on a milk diet, but complete cure resulted. Shipley[23] insists on an important practical point in the treatment of the anæmia of scurvy, namely, that copper should not be given with vitamin C because its action is to destroy that vitamin.



GRAPH IX.—Effect of orange juice on scorbutic anæmia.

The existence of an anæmia due to the absence of vitamin C, but occuring before frank symptoms of scurvy become manifest (pre-scorbutic anæmia), has been insisted upon by Weill and Mouriquand[24] who have described the existence of anæmia which resisted treatment by iron but which was cured by lemon juice. Recently Rohmer and Bindschelder[22] investigated a group of 22 anæmic infants with a view to determining the existence of this ' pre-scorbutic anæmia.' In six of these cases the administration of iron did not produce any effect, but when vitamin C was given in addition to the iron a prompt cure resulted. They suggest that it is possible that vitamin C has some effect on the metabolism of iron, and that in regard to iron it plays a rôle similar to that played by other vitamins in relation to certain elements of the diet. A simpler and quite adequate explanation of the observed facts is, however, that more than one factor may be lacking in some cases of deficiency anæmia; for instance, a child suffering from nutritional anæmia may also develop scurvy and the anæmia of scurvy, in which case for its complete cure vitamin C as well as iron will be required.

4. Anæmia of cœliac disease.

The anæmia of cœliac disease is in reality one of that important group of deficiency symptoms which are so characteristic of that disorder and is of peculiar interest amongst deficiency anæmias in that not only is it manifested as hypochromic anæmia similar to those already discussed, but also in some cases as a hyperchromic megalocytic anæmia.

In his original description of the 'cœliac affection' Gee[25] wrote:—'Cachexia, a fault of sanguinification betoken by pallor and a tendency to dropsy, is a constant symptom: the patients become white and puffy'; and although the symptom is not by any means constant some degree of anæmia of the hypochromic type is not infrequently present. The hæmatological picture differs in no way from that found in the nutritional anæmia of infants and like theirs it is usually an iron, or iron and copper, deficiency anæmia. There is nothing characteristic about it except that sometimes scurvy may be an element in its production.

Recently, however, Strauss and Castle, and also Bennett, Hunter and Vaughan[26] have drawn attention to the existence of a hyperchromic megalocytic anæmia in cœliac disease. This form of anæmia is of great rarity and according to the latter authors is indistinguishable from that found in sprue, in tropical megalocytic anæmia, and in association with gastro-colic fistula, but differs from Addisonian pernicious anæmia. The points of difference are that in the first group the indirect van den Bergh reaction is low, there is a striking absence of poikilocytosis, free hydrochloric acid is frequently present in the gastric juice, or may return on treatment, and the anæmia responds to marmite; whereas in pernicious anæmia the indirect van den Bergh reaction is high, poikilocytosis is marked, free hydrochloric acid is rarely present in the gastric juice and does not return on treatment, and marmite has no therapeutic value. The statement that marmite is ineffective in pernicious anæmia requires some modification in view of recent work which shows that it is of value particularly as a maintenance measure.

In order that the maturation of the megaloblast to the normoblast may occur it is essential to supply the bone marrow with the hæmatinic factor which is produced by the interaction of an intrinsic factor present in normal gastric juice with an extrinsic factor. The maturation will not take place, therefore, unless the intrinsic factor is present in the gastric juice; the extrinsic factor is supplied in the food and the resulting hæmatinic factor is absorbed. Strauss and Castle now hold that the extrinsic factor is closely related to vitamin B_{12}, if not that vitamin itself. Now marmite is rich in this vitamin and its efficacy in preventing or curing the megalocytic anæmia of cœliac disease is due to the fact that this form of anæmia will occur when lack of the extrinsic factor has prevented the specific reaction taking place.

From a view of the literature Bennett, Hunter and Vaughan accept 21 cases, including two of their own, as examples of hyperchromic megalocytic anæmia associated with a condition which was probably cœliac disease. Of these only five cases occurred in children of twelve years or under, the actual

ages being $1\frac{1}{2}$, 3, $4\frac{1}{2}$, 9 and 12 years. The ages of the other cases varied from 16 to 62 years. Of the five cases described in children three (aged 3, $4\frac{1}{2}$, and 12 years) seem to be above criticism, but in the other two cases the colour index was considerably below unity. It will be seen, therefore, that in children this form of anæmia is of the greatest rarity. It seems to us that a possible explanation of this rarity is that two conditions are necessary for its development, namely:—first, the cœliac disease must be of long-standing; secondly, its treatment must have been inadequate. If the case records of megalocytic anæmia in cœliac disease are examined they will be found to support this explanation, because the majority have occurred in adults whose clinical history shows that they have suffered from cœliac disease for years, in some instances from childhood, and that they have not received adequate treatment. For instance, we have seen one typical example of this form of anæmia in a woman, aged 23, who at the age of 10 years had obvious symptoms of cœliac disease, but had never adequately carried out the dietetic treatment advised. It may perhaps be added that the term ' adequate treatment ' includes the administration of marmite or some form of dried yeast which we have regarded for some years as an essential part of the treatment of cœliac disease. If time really be a factor in the development of this megalocytic anæmia it should be possible to trace the transition from the hypo- to the hyper-chromic type of anæmia; indeed Witts has said that if a fatty diarrhœa persists for more than a few months an anæmia always develops which tends to pass through three phases, (a) hypochromic anæmia with a normoblastic marrow, (b) megalocytic anæmia with a megaloblastic marrow, and (c) aplastic anæmia; and he has quoted a statement of Fanconi to the effect that a child with cœliac disease may pass through these three phases.

There is yet another variety of anæmia which has been described in cœliac disease, namely an erythroblastæmia resembling Cooley's anæmia. Bennett, Hunter and Vaughan have found this form in two adults suffering from cœliac disease who also showed in mild degree the bone changes and facial characteristics described by Cooley. These are the only cases on record and they differ from the form described by Cooley in that they responded to treatment with iron, whereas all forms of treatment have been ineffective in the cases of Cooley's anæmia in childhood so far recorded.

For the purpose of this paper we have reviewed 19 cases of cœliac disease which have come under our observation during the last few months; 14 of them were in the initial stages of their treatment, and the remaining 5 had been under treatment for a more lengthy period. The ages of the children varied from 18 months to 10 years, 9 of them were free from anæmia, 6 showed anæmia of the hypochromic type, 3 a mild degree of hyperchromic anæmia, and in one the blood showed a condition approximating to a hyperchromic megalocytic anæmia. Splenomegaly sometimes occurs in cœliac disease, a fact originally pointed out by Gee; it has not been present in this series and we have only met it twice in over a hundred cases of the disease.

Hypochromic anæmia.—It has already been stated that this anæmia is in all respects identical with the ordinary nutritional anæmia of infants. The statement is borne out by a reference to the following case:—

Case 3.—F. A., male, aged 2 years, who died of influenza. Below are shown the results of the examinations of the blood over a period of nine months. The anæmia is microcytic, and in Fig. 2 is shown the Price-Jones curve made from a blood film of this child taken a week or two before death. This boy did not receive any iron during the whole course of his illness.

Date.	31.3.32.	3.10.32.	5.12.32.	19.12.32.
Red blood cells	4,404,000	3,907,000	4,520,000	4,160,000
Hæmoglobin per cent.;	60	43	42	44
Colour index	0·68	0·55	0·47	0·53
Leucocytes	11,350		17,400	16,750
Reticulocytes per cent. of red cells	1·0	0·8	0·7	3·1
Platelets	plentiful			
Neutrophil polymorph.	19·5		22	
,, metamyelocytes ...	·5			
Large lymphocytes	74·5		50	
Small lymphocytes	4·5		24	
Monocytes	1·0		4	
Normoblasts	1		1	
Anisocytosis	+		++	+++
Polychromasia	+			++
Eve's halometer reading				7·25μ

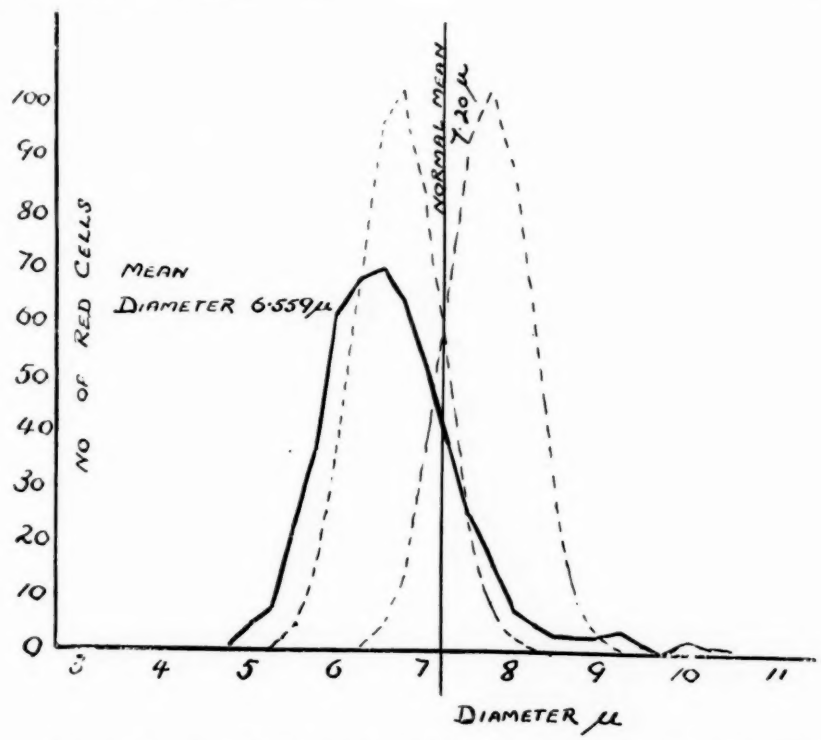


Fig. 2.—Case 3. Price-Jones curve showing the microcytic character of the hypochromic anæmia of cœliac disease.

Autopsy (26 hours after death, December). The shaft of the femur was thinned. Bony trabeculæ were few and thin. The marrow was red throughout; there was a well-defined patch of ' red currant jelly ' at its lower end and some ill-defined patches were scattered elsewhere.

Histologically low-power magnification of the section (which was stained by hæmatoxylin and eosin) showed a structureless matrix, staining pink, in which were scattered small aggregations of red cells like minute hæmorrhages into the matrix, and also small foci of hæmatopoiesis. Higher magnification (Fig. 3) showed that these foci consisted of granular myelocytes and metamyelocytes with a fair proportion of young nucleated red cells. These latter were usually about 5μ with nuclei 4μ in diameter which practically filled the cells and stained deeply. A minority showed stippling, but the majority were rather of normoblastic type. More mature nucleated red cells with small nuclei were rare. Rather prominent endothelial cells with pale oval nuclei were scattered through the groundwork. In the more cellular areas, the erythroblasts were very numerous, and the myeloid cells were normal except that polymorphonuclear cells were scanty. Smears of the cellular areas of marrow showed cells, which generally stained badly; normoblasts, myelocytes and metamyelocytes were present, but definite megaloblasts were not found. Smears from the jelly-like areas showed normoblasts, normoblastic nuclei and rather large

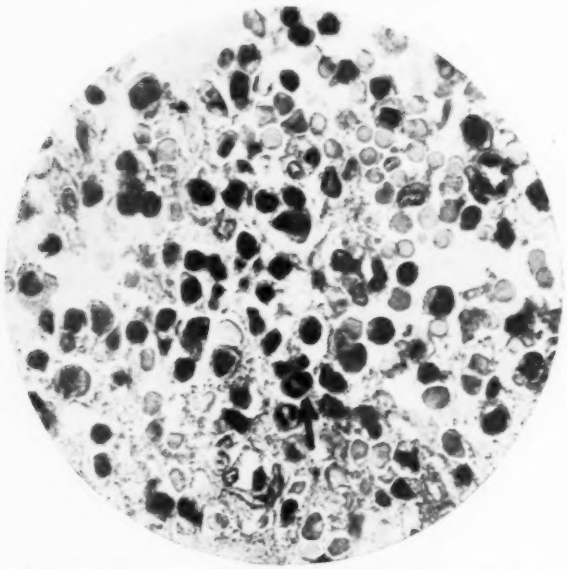


Fig. 3.—Case 3: cœliac disease; marrow × 700; showing erythropoietic focus; cells almost entirely normoblasts; mitosis is seen (arrow).

pale cells which were possibly endothelial elements. The outstanding features of these histological appearances were (1) the replacement of a large part of the marrow by structureless jelly-like material; (2) the presence of erythropoiesis, in which the cells were generally rather immature, although definite megaloblasts were not seen; (3) slight immaturity of the myeloid series.

Reference has been made to the fact that the hypochromic anæmia of cœliac disease is the result of an iron, or an iron and copper, deficiency. This deficiency is largely due to the disturbance of absorption which is so characteristic of cœliac disease, but it may also be due to lack of mineral supply and this is particularly the case during those periods when protein skimmed milk is the only food taken by the child. Some of the difficulty in absorption may be due to gastric hypo-acidity because achlorhydria is not uncommon in cœliac disease although we have never failed to obtain free

hydrochloric acid by subcutaneous injection of histamine. In all cases of cœliac disease it is quite as essential to supply iron in an available form, and in sufficient quantity as it is to supply the various vitamins. We have found that hypochromic anæmia responds very quickly to ferrous sulphate given in adequate doses, and that associated with the improvement in the anæmia there is also a marked improvement in the general condition of the child.

An example of the results obtained with this drug is given here: ferrous sulphate in doses of 12 grains daily were given, commencing on November 13th, 1932, to a child suffering from the hypochromic anæmia of cœliac disease.

Date.	14.11.32.	21.11.32.	23.11.32.	1.12.32.	5.2.33.
Red blood cells ...	5,340,000		6,000,000	5,440,000	5,300,000
Hæmoglobin per cent.	54		63	75	100
Colour index	0·41		0·53	0·69	0·94
Reticulocytes per cent. of red cells	1·01	5·1	3·75	3·6	
Punctate basophilia ...				+	
Polychromasia	+		++	++	
Anisocytosis	+		++	++	

Hyperchromic anæmia.—Three children showed a mild degree of hyperchromic anæmia and two of these were particularly interesting, because the administration of marmite failed to produce a normal blood picture.

Case 4.—P. W., female, aged 18 months, was treated in the routine way for cœliac disease, which included the consumption of ½ oz. of marmite specially prepared to remove some of the salts. After she had taken the marmite for one month an examination of her blood gave the following results:—red blood cells 3,591,000 per c.mm.; hæmoglobin 76 per cent.; colour index 1·05; reticulocytes 1·0 per cent. of red cells.

It had occurred to us that the cause of cœliac disease itself might possibly lie in the absence of some intrinsic factor in the gastric juice or more probably in the intestinal juices because the metabolic defect is one of absorption. We therefore tried the effect of giving to children suffering from this disease the dessicated mucosa of hog's stomach or of hog's duodenum and jejunum in addition to the routine administration of marmite. So far we have not obtained any improvement in the hypochromic anæmia nor any striking improvement in absorption, but an interesting thing happened when dessicated gastric mucosa was given in Case 4, in that three weeks later an examination of her blood showed that it was normal. The red cells were 5,000,000 per c.mm., the hæmoglobin 90 per cent., and the colour index 0·9.

Case 5.—D. B., female aged 2½ years, was admitted into hospital under the care of our colleague, Dr. J. M. Smellie, to whom we are indebted for permitting us to record the case. She was given 3 drm. of marmite daily, and 12 days later an examination of her blood gave the following results:—red blood cells 4,370,000, white blood cells 6,850 per c.mm.; hæmoglobin 88 per cent.; colour index 1·0; reticulocytes 0 per cent. of red cells; well-marked anisocytosis and megalocytosis. A recent

examination of the blood, four months after starting the continuous administration of marmite in the amounts stated showed that the degree of anæmia had increased and that it was still of the megalocytic type, the actual figures being:—red blood cells 3,500,000, leucocytes 7,350 per c.mm.; hæmoglobin 68 per cent.; colour index 0·97; reticulocytosis less than 0·5 per cent. of red cells; well-marked anisocytosis and a degree of polychromasia. The Price-Jones curve (Fig. 4) made at this time demonstrates quite clearly the anisocytosis and the megalocytosis, the mean diameter of the red cells being 7·905μ whereas the largest mean diameter within normal limits is 7·718μ (Price-Jones).

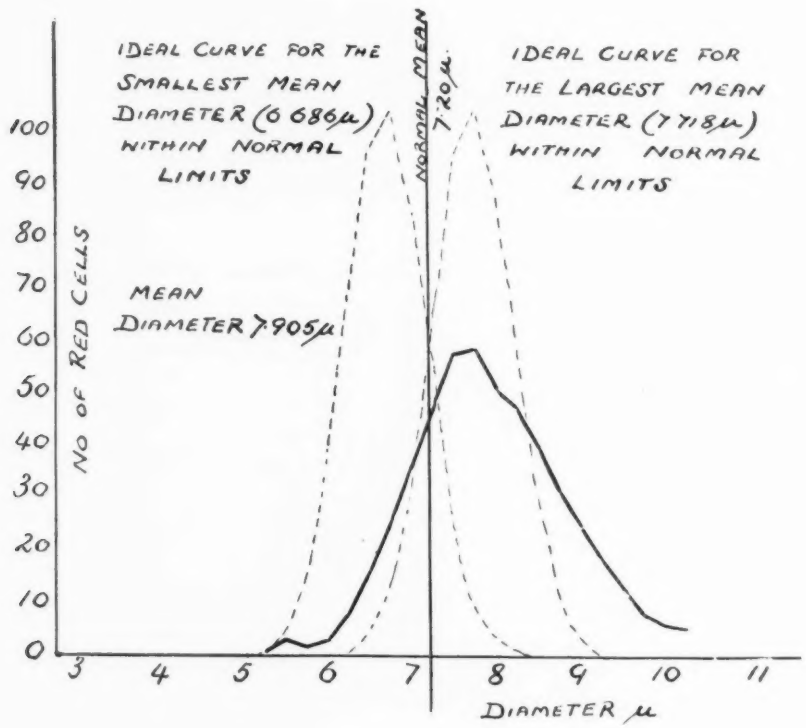


FIG. 4.—Case 5. Price-Jones curve of a case of megalocytic anæmia of cœliac disease.

These two cases suggest very strongly that some children with cœliac disease are lacking in Castle's intrinsic factor. An objection might be raised that the extract of marmite in our first case did not contain vitamin B. This objection cannot be sustained for the following reasons:—First, the vitamin-B complex is soluble in alcohol, but vitamin B_1 is insoluble in 92 per cent. and B_2 insoluble in 70 per cent. alcohol and upwards[27]. The extract used is prepared by shaking marmite with 65 per cent. alcohol for some hours, filtering and evaporating the filtrate; the resultant residue is then made up with water in such a way that one fluid ounce represents one ounce of marmite. Secondly, extracts prepared in this way have proved effective in curing two children suffering from pellagra. In any case this criticism cannot apply to the second child who received untreated marmite. This

child has not yet been given dessicated gastric mucosa but we anticipate that this will prove effective.

The importance of these observations is increased by the fact that Castle and Rhoads[1] state that there is a lack of intrinsic factor in some cases of sprue. Of course it is possible that the child (Case 4) was not lacking in intrinsic factor but that during the period she was taking gastric mucosa the absorption improved, so that the product of the interaction of intrinsic and extrinsic factors which had previously been indifferently absorbed was absorbed in sufficient amounts to produce a normal blood picture. Such an explanation might also hold in the cases of sprue described by Strauss and Castle, a possibility which doubtless was considered by them.

Case 6.—D. H., aged 2 years, showed a mild anæmia with a colour index of nearly unity (0·97) in the only examination carried before death. At autopsy the bone marrow was found to have a typical red-currant jelly appearance. The replacement of the normal marrow by the jelly-like material was more extensive than in the fatal case of hypochromic anæmia (Case 3) described above, and occupied the whole shaft of the femur. The histological features of the marrow were similar in the two cases except that in this instance the hæmatopoietic areas were more minute and more difficult to recognize. Many of these appeared to be composed of endothelial cells and were scattered widely in the matrix.

5. Other forms of hyperchromic megalocytic deficiency anæmia.

There are two other forms of hyperchromic megalocytic deficiency anæmia which may occur in childhood, namely:—pernicious anæmia and Bothriocephalus anæmia. Both are of extreme rarity.

The question whether pernicious anæmia ever occurs in early life has been a subject of debate, since the majority of the cases reported are unconvincing, some being clearly examples of acute hæmolytic anæmia. We have no clinical observations of our own to offer as evidence for or against the existence of pernicious anæmia in children, but we would insist that before an anæmia can be classed as Addisonian pernicious anæmia it must show certain features in addition to megalocytosis: these are:—(1) a high indirect van den Bergh reaction; (2) marked poikilocytosis; (3) almost always absence of free hydrochloric acid in the gastric juice; (4) a reticulocytosis with liver extract. In 1928 H. K. Faber[28] described a case of anæmia in an infant 9½ months old which fits these criteria more closely than any other instances in the literature.

Bothriocephalus anæmia is almost unknown in the first decade of life, only three cases having been recorded.[29] The clinical manifestations do not differ from those in adults, and the anæmia is probably due to the destruction by the worm of the hæmopoietic product of the interaction of the extrinsic and intrinsic factors, or to its diminished absorption as the result of the action of the worm on the intestinal mucosa.

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COPPER IN THE TREATMENT OF NUTRITIONAL ANÆMIA IN INFANCY

BY

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Ample evidence now exists to show that the nutritional anæmia, or milk anæmia, of babies bears a very close resemblance to the milk anæmia of rats, but whereas young rats in whom anæmia is induced by prolonged milk feeding suffer from a deficiency of copper as well as of iron, and require both copper and iron for their cure[1], it is not yet proven that copper deficiency plays any part in the ordinary case of nutritional anæmia in the human infant. This study was undertaken in the hope of providing further evidence on this question.

The mode of action of copper.—The actual mode of action of copper is unknown, but recent experimental work has thrown some light on the question. Elvehjem, Steenbock and Hart[1] state that it is not a constituent of the hæmoglobin molecule. In the absence of copper, iron supplied to young rats by mouth is absorbed from the intestine, and is stored chiefly in the liver, as Josephs has shown[6], but is not used for building hæmoglobin. If copper is given it renders this iron utilizable for hæmoglobin formation: ' copper does not affect the assimilation of iron, but does function in the conversion of inorganic iron into hæmoglobin '.[2]

Görter, Grendel and Weyers[3] have shown that in a small series of children suffering apparently from nutritional anæmia, the copper content of the blood was more than twice as high as in the new-born and markedly higher than in control convalescent children, a fact which suggests (a) that in these cases of anæmia there was no shortage of copper, and (b) that the body's need for hæmoglobin calls out copper into the circulation.

The response to treatment with an iron salt.—The author[8] has already shown that iron and ammonium citrate will cure the vast majority of cases of nutritional anæmia in babies in London provided there is no serious infection present[9]. In some infants the response is very slow, but sometimes in such instances there will be found slight pyrexia, presumably from an undiscovered infection which is retarding cure. The iron and ammonium citrate successfully used in this investigation contained, however, a trace of copper, 1·7 mgrm. per 100 grm.[10], and thus left open the possibility that the trace of copper was a material factor in the results obtained. This hypothesis

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nevertheless seems improbable, as the copper added to the diet by the iron salt amounted to a very small percentage of the total copper intake. Even when the babies were entirely bottle fed, and so getting food especially low in copper, the increase in copper due to the iron salt would only amount to, say, 4 per cent. It is not easy to be convinced that a rise in copper intake from 1·26 mgrm. to 1·31 mgrm. weekly, which is the approximate figure for a baby in our series getting 1½ pints of milk daily, is likely to have been a material factor in the cure.

If the deficiency in babies suffering from nutritional anæmia is generally a combined deficiency of both iron and copper, one might anticipate a better response to treatment with an iron salt in those receiving more copper in their food than in those receiving much less. Babies on a mixed diet get much more copper than those on cow's milk alone, but iron and ammonium citrate appears to cure at about the same rate whether the baby is entirely bottle fed or is given mixed food[5, 11]. This fact might be used as an argument against a deficiency of copper in these babies, but here we seem on uncertain ground. There is no doubt that iron deficiency is present, yet in a clear case of nutritional anæmia inorganic iron, in doses which are vast compared with the total iron of the body, produces a better response than does the organic iron present in an ordinary mixed diet containing eggs, meat, green vegetables, etc.[12] Hence if inorganic iron in large doses is the best method yet known of making good an iron deficiency, it is possible that inorganic copper, also in relatively large doses, might be the best method of making good a copper deficiency. So that this argument against a copper deficiency, because a similar rate of response is obtained with iron whether the food copper is low or not, seems by itself inconclusive.

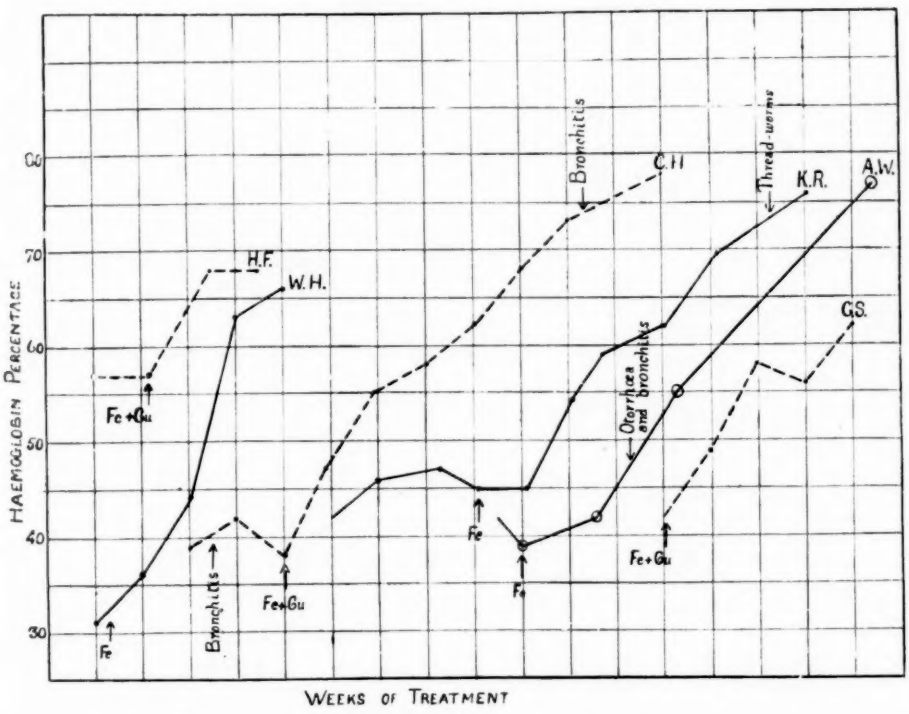
The influence of copper.—During the last few years a number of articles have been published on the treatment of anæmia in the human subject by the administration of copper. At least one of these was based on a series of one hundred children treated with iron and copper, but unfortunately few of the papers give sufficient data to allow the reader to reach any conclusions as to the value of copper therapy in the nutritional anæmia of babies.

On the other hand, Josephs has carried out at the Johns Hopkins Hospital some interesting and valuable work on anæmia in babies, and his cases have been accepted by many as proof that in nutritional anæmia of infancy copper and iron administered together produce a more rapid rise in hæmoglobin than iron alone[5]. However, the majority of the cases quoted in his paper were suffering from, or had begun to be convalescent from, some serious infection, such as pneumonia, empyema, tuberculosis or syphilis; and some at least were presumably receiving other forms of treatment in addition, which makes it difficult to draw any clear cut conclusions; for he himself, in another important paper[7] demonstrates the blood destruction occurring during severe infections, and the variations that exist in the subsequent tendency to ' spontaneous cure.' The number of Josephs' published cases

(including controls and cases treated with copper), which were uncomplicated by any serious infection was 6, starting treatment at hæmoglobin levels ranging between 30 and 50 per cent. Though these cases certainly suggest that the copper supplement (copper sulphate) may have accelerated cure of the anæmia when given in addition to iron (iron and ammonium citrate), yet the number of cases is too small to be conclusive (only 3 were given iron plus copper throughout the time of treatment) in view of the great individual variations in response to iron and ammonium citrate without added copper. Moreover, some of Josephs' cases were first given iron alone and later iron

GRAPH I.

Results of treatment (for Cases see p. 153).



Iron and ammonium citrate plus copper sulphate - - - - - - - - - -

Iron and ammonium citrate alone .————————.

Saccharated ferrous carbonate o————————o

plus copper. In the experience of the present author not only do some babies respond with a more rapid rise than others to iron, even when the initial hæmoglobin levels correspond, but the individual baby often does not show an even rise (see Graphs I and II). The rise with iron treatment tends to be most rapid when the initial hæmoglobin level is low, and, as Josephs[5] and others have pointed out, in many cases the rise tends to be slower and more irregular as the hæmoglobin level approaches normal. On the other hand, occasionally a child may show little response to iron treatment for a month or even more, and subsequently his hæmoglobin may rise rapidly. Hence to

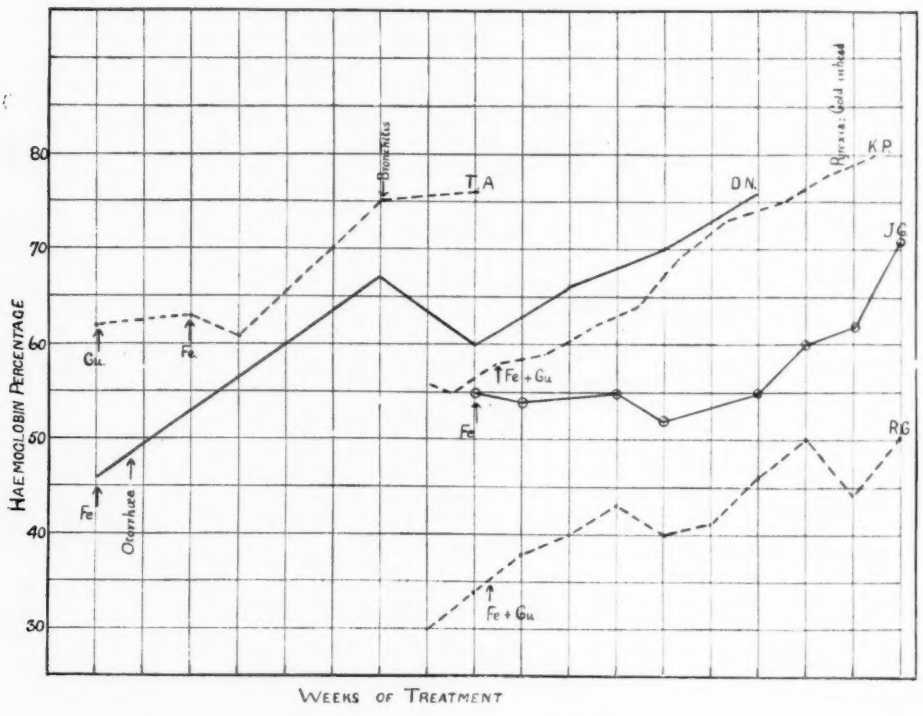
compare a child's initial response to iron to its subsequent improvement on iron plus copper seems likely to lead to an erroneous conclusion. Since Josephs does not state that his iron salt was copper-free, the presumption is that it, like most other iron salts on the market, including that used by the present author, contained traces of copper.

Present investigations.

To demonstrate satisfactorily that iron and copper together produce a better response than iron alone in nutritional anæmia, it seems desirable to compare a series of similar babies treated throughout with both minerals

GRAPH II.

Results of treatment (for Cases see p. 153).



Iron and ammonium citrate plus copper sulphate - - - - - - - - - -
Iron and ammonium citrate alone .————.
Saccharated ferrous carbonate o————o

with a series treated with iron alone, eliminating as far as possible any complicating factors, such as intercurrent illness, etc. This the author has attempted, but with only partial success. As everyone who has attempted such work is aware, there are various difficulties to be overcome, and the observations here described are not in themselves conclusive, though it is hoped that taken in conjunction with other work they may help to clarify the problem.

There were certain advantages in using iron in the form of iron and ammonium citrate, because the use of this salt in most of her previous work on nutritional anæmia had rendered the author familiar with the type of response to be expected from it. Failing, however, to get iron and ammonium citrate free from traces of copper, the salt used was obtained from the same firm as before, the British Drug Houses. Both groups of children were given this iron salt, and the ' copper group ' received in addition a supplement of copper sulphate. If the babies under observation suffered from copper deficiency then more rapid cure should be obtained by giving iron with an ample copper supplement, than by giving iron with only a trace of copper impurity which could scarcely make good an existing deficiency.

The second difficulty was to obtain sufficient numbers of uncomplicated cases and to ensure that they received regular treatment. In the author's iron work regular administration of the iron salt could be obtained in out-patients because the iron was incorporated in dried milk, whereas in this work, done on a small scale, the copper was given in the form of a mixture. Hence whenever possible it was desirable to treat the babies as in-patients. The great susceptability of anæmic infants to infections of all sorts renders their in-patient treatment difficult, and a number of cases had to be excluded because they suffered from infections, while others were discharged for their own safety, before any result was obtained, on account of ward infection.

Dosage of iron and ammonium citrate.—The dosage of iron used in the cases illustrated in this paper varied between $4\frac{1}{2}$–9 grn. of iron and ammonium citrate daily. In every case, however, the iron was begun gradually, so that the amount given in the first week amounted to considerably less than this quantity.

Dosage of copper sulphate.—Except for one of the cases (H.C., Graph II), the copper supplement was one-twentieth of a grain of copper sulphate ($CuSO_4$, $5H_2O$) daily, divided into three doses. This also was begun gradually. This dose of copper sulphate is smaller, weight for weight, than the dose given by Hart and his colleagues at Wisconsin to their rats. These workers found 0.05 mgrm. of copper as copper sulphate, given on six days in the week, a satisfactory supplement for a young rat, and that 0.01 mgrm. of copper would produce cure, though more slowly. They calculated[4] that 0.05 mgrm. of copper increased the copper intake say six-fold. If we assume that the young rat's weight is about 60 grm. then weight for weight the amount needed by a 14-lb. baby would be about one-fifteenth to one-third of a grain of copper sulphate daily. But when one remembers that the rate of increase in weight of young rats is so much more rapid than that of babies it seems probable that the copper needs of babies would be relatively less. The dose actually used by the author, as stated above, was one-twentieth of a grain of copper sulphate daily. The doses given by Josephs varied with the child's weight. For babies of 14 lb. his doses were about ten times as large as the author's, or about $\frac{1}{2}$ grain of copper sulphate daily ($\frac{1}{4}$ to $\frac{1}{2}$ grain is given as the ' average ' adult dose by Martindale & Westcott

in the Extra Pharmacopœia). Josephs' dosage was, he says, well tolerated in babies, but the progressively larger amounts for older and heavier children sometimes caused diarrhœa and vomiting.

One case illustrated in Graph II (R.C.) received a much smaller dose of copper than the rest of the cases shown, namely a dose of 0.12 mgrm. copper sulphate (or about 0.03 mgrm. Cu) daily, an amount which approximately trebled the copper addition to the diet provided by the traces of copper in the iron and ammonium citrate. This was given with the idea that if the traces of copper in the iron salt used were playing any part in the results obtained, then trebling this trace should intensify the effect.

Dosage of saccharated ferrous carbonate. As a matter of interest two cases given saccharated ferrous carbonate are also shown on the charts. The dosage of saccharated ferrous carbonate, $4\frac{1}{2}$-9 grains daily, was, as regards iron content, similar to that of the iron and ammonium citrate given. Iron and ammonium citrate contains approximately 20 per cent. of iron, and the saccharated ferrous carbonate, say, 25 per cent.

Results.—The number of uncomplicated cases treated with the copper supplement in this series is much too small to warrant the calculation and comparison of the average rates of response to the two methods of treatment, though this would undoubtedly be the method of choice were the numbers large enough. However, since they are not, the individual responses to treatment with iron and copper combined are compared with typical responses to treatment with iron and ammonium citrate alone. Several of the babies suffered from minor infections (e.g., nasal discharge and bronchitis), but all children with severe infections have been excluded.

In Graph I is shown the best response obtained with iron and ammonium citrate plus the copper supplement, a rise of 40 per cent. in 56 days (C.H.); and with it two examples (W.H. and K.R.), of good response with the iron and ammonium citrate alone, in one case a rise of 35 per cent. in 24 days (W.H.), and in the other a rise of 31 per cent. in 49 days (K.R.). For purposes of comparison one case (A.W.) on saccharated ferrous carbonate is included, with a rise of 38 per cent. in 53 days. It will be seen that all four cases show a response of the same order; the baby who has the lowest initial hæmoglobin showing the most rapid rise in hæmoglobin level. There is not one case among those given the copper supplement that cannot be matched, or even bettered, by cases treated with iron and ammonium citrate alone.

Graph II (R.C.) shows the poorest response obtained with iron and copper in an apparently uncomplicated nutritional anæmia. This was a very anæmic baby (30 per cent. hæmoglobin) with a slow and irregular rise of only 16 per cent. in 59 days, although there was no evidence of any microbic infection. This infant received the very small copper addition mentioned above, and the response was unusually poor for a child given iron and ammonium citrate.

The other curves on the two charts (Graph I, H.F. and C.S.; Graph II, T.A. and K.P.) show intermediate results. J.C. shown in Graph II, who was given saccharated ferrous carbonate, is included because of his very long

' latent period.' No evidence of infection was present and he was known to have regular treatment. There was no rise in hæmoglobin for 6 weeks, and thereafter a fairly rapid rise: 16 per cent. in 21 days, without change of treatment. In such a case if copper had been added during this long ' latent period ' one would have been inclined to attribute the subsequent rise to the new factor.

Discussion and conclusions.

The comparison of cases treated with and without the copper supplement does not suggest that copper was of any benefit in this type of anæmia: in fact, the types of response obtained with copper were very like those customarily seen with iron and ammonium citrate alone. From which it seems fair to conclude that the deficiency affecting these babies was a deficiency of iron and not of iron plus copper. The successful results of treatment with a copper-free salt published in the present number of this journal by Professor Parsons and his colleagues lead to the same conclusion and are of great importance.

The facts set forth when taken together indicate, in the author's opinion, that copper deficiency does not play any important part in the common nutritional anæmia of babies in this country. Such negative results, however, do not of course show that copper deficiency never occurs in babies suffering from iron deficiency. It is possible, for example, that a baby might be born with insufficient copper as the result of a maternal dietetic deficiency, and so develop a nutritional anæmia due to both copper and iron deficiency. However, iron salts with only minute and presumably insignificant traces of copper have been found effectual in the treatment of this anæmia in many countries of the world, and the line of argument used in this paper would indicate that, in other regions as in London, copper deficiency in babies is uncommon.

When considering possible copper deficiency the varying copper content of milk is of considerable interest. Human milk being richer in copper than cows' milk, it can be assumed that copper deficiency is more likely to occur in bottle-fed babies. Another point of importance is the copper content of ordinary fluid cow's milk on the market as compared with the copper content of milk obtained under special precautions to prevent any copper contamination. It appears that copper contamination is extremely common in milk and will materially increase its percentage of copper. Thus if milk is pasteurized in contact with copper the following comparative values have been obtained:—

Quam & Hellwig: before pasteurization: 0.26-0.52 mgrm. Cu per litre; after pasteurization: 0.60-0.70 mgrm. Cu per litre; and

Görter, et al.[3]: before pasteurization: 0.09-0.14 mgrm. Cu per litre; after pasteurization: 0.17-0.29 mgrm. Cu per litre.

In the dried milk used for the control cases in our work on infantile anæmia[8] estimations of copper in two samples gave 0.19 mgrm. Cu (Professor

E. B. Hart), and 0.25 mgrm. Cu (Dr. F. Carr) per litre of reconstituted milk, as compared with a value of 0.123-0.184 mgrm. Cu per litre for fresh milk, as estimated by Elvehjem, Steenbock and Hart by the same technique. Hence it is possible that the copper contamination of cow's milk given to babies may actually be a factor of importance in preventing copper deficiency.

The fact that large doses of iron salts are desirable in the treatment of anæmia has by some been explained on the hypothesis that the traces of copper present in these salts are of fundamental importance, but if the conclusions here set out are correct, then as regards nutritional anæmia in babies, at any rate, this hypothesis falls to the ground.

Summary.

The part played by copper in the common nutritional anæmia of infancy is discussed, and the response in individual infants to treatment with iron and ammonium citrate plus copper sulphate, is compared with the response to iron and ammonium citrate alone.

The conclusion reached is that although copper deficiency may occur in isolated cases of nutritional anæmia it plays no part in the great majority of cases seen in London, and that this probably holds good for other parts of the world.

The author offers her thanks for their help and co-operation to the resident medical staff and nursing staff of the Queen's Hospital for Children, and especially to Miss E. K. Wackett, the ward sister who had charge of the majority of the children treated with iron and copper.

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SYNOPSIS OF CASES SHOWN IN GRAPH I.

Cases treated with iron and ammonium citrate plus copper sulphate.

H. F. Birth weight 7½ lb. Age 13 months; well nourished; weight 19 lb.; evidence of healed rickets. Given a mixed diet. Anæmia treated with iron and amonium citrate and copper sulphate. Hæmoglobin rose from 57 to 68%, i.e., 11%, in 16 days. Child treated as in-patient. Gained 2 lb. 2 oz. in 17 days. Discharged on account of infection in ward.

C. H. Twin, birth weight 4 lb. Age 13 months; very undersized; weight 12 lb. 9¾ oz. Suffering from rickets and anæmia, and a persistent nasal discharge. Fed on milk and cereal only until admission. Put gradually on to a mixed diet and cod liver oil. Anæmia treated with iron and ammonium citrate and copper sulphate. Hæmoglobin rose from 38 to 78%, i.e., 40%, in 56 days. Child treated as in-patient. Weight remained almost stationary during this period.

C. S. Birth weight unknown, said to be premature. Age 10 months; undersized, rather thin; weight 11 lb. 10¾ oz. Bottle fed till admission. Put gradually on to a mixed diet. Anæmia treated with iron and ammonium citrate and copper sulphate. Hæmoglobin rose from 42 to 62%, i.e., a rise of 20%, in 28 days. Child treated as in-patient. Gained 11 oz. in one month.

Cases treated with iron and ammonium citrate alone.

W. H. Twin, small at birth. Age 16 months, weight 14 lb. 14 oz. Given a mixed diet. Anæmia treated with iron and ammonium citrate alone. Hæmoglobin rose from 31 to 66%, i.e., 35%, in 24 days. Child treated as in-patient.

K. R. Birth weight 6 lb. 15 oz. Age 2 years and 9 months; well nourished but small for age; weight 25 lb. 6 oz.; suffering from rickets. Given a mixed diet and cod liver oil. Anæmia treated with iron and ammonium citrate alone. Hæmoglobin rose from 45 to 76%, i.e., 31%, in 49 days. Child treated as in-patient at first, subsequently as out-patient, and gained 2 lb. 6½ oz. in 50 days.

Case treated with saccharated ferrous carbonate.

A. W. Birth weight unknown. Aged 15 months. Well nourished; weight 20 lb. 14 oz.; mentally backward. Given a diet consisting chiefly of milk and cereal with some fruit and vegetable daily. Anæmia treated with saccharated ferrous carbonate. Hæmoglobin rose from 39 to 77%, i.e., a rise of 38%, in 53 days, in spite of an attack of double otorrhœa. Child treated as in-patient, gained 2 lb. 1 oz. in 53 days.

SYNOPSIS OF CASES SHOWN IN CHART II.

Cases treated with iron and ammonium citrate plus copper sulphate.

T. A. Birth weight 6 lb. Age 11 months. Fairly well nourished, very flabby; weight 18 lb. 1 oz. Given mixed diet. Anæmia treated with iron and ammonium citrate and copper sulphate, the copper sulphate was begun two weeks before the iron. Hæmoglobin rose from 63 to 76%, i.e., 13%, in 42 days after iron started. Child treated as out-patient. Gained 12 oz. in this period.

K. P. Birth weight 8 lb. Age 20 months. Very small and thin; weight 13 lb. 8 oz.; suffering from severe active rickets. Diet up to time of admission was dried milk and orange juice. Put gradually on to a mixed diet, and given calciferol.

Anæmia treated with iron and ammonium citrate and copper sulphate. Hæmoglobin rose from 58 to 80%, i.e., 22%, in 56 days. Child treated as in-patient. Gained 2 lb. 14 oz.

R. C. Birth weight 6 lb. 4 oz. Age 11 months. Nutrition poor; weight 15 lb. 4 oz.; history of a recent attack of enteritis. Fed on dried milk till admission. Put gradually on to a mixed diet. Anæmia treated with iron and ammonium citrate and copper sulphate (very small dose of copper, see text). Hæmoglobin rose from 34 to 50%, i.e., 16%, in 59 days. Child treated as in-patient, and gained only 6 oz. in that time, though there was no evidence of any infection until he developed measles at the end of the period of observation.

Case treated with iron and ammonium citrate alone.

D. N. Twin, birth weight 3½ lb. Age 7½ months. Well nourished, weight 15 lb. 2 oz. Fed on dried milk till admission and was changed gradually on to a mixed diet. Anæmia treated with iron and ammonium citrate. Hæmoglobin rose from 46 to 76%, a rise of 30%, in 91 days. Treated first as an in-patient, later as an out-patient; gained 5 lb. 1½ oz. in this period.

Case treated with saccharated ferrous carbonate.

J. C. Birth weight unknown: said to be premature. Age 16 months. Well nourished; weight 20 lb. 0½ oz.; suffering from rickets. Given diet consisting chiefly of milk and cereals with some fruit and vegetables daily and cod liver oil. Anæmia treated with saccharated ferrous carbonate. Hæmoglobin almost stationary for first 6 weeks at about 55%, thereafter rose to 71%, i.e., by 16%, in another 21 days, without change in treatment, making a total rise of 16% in 63 days. Child treated as an in-patient, gained 1 lb. 11½ oz. in 63 days.

A CASE OF MEGADUODENUM WITH MULTIPLE ANOMALIES OF THE ILEUM

BY

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A chronic dilatation of the duodenum is sometimes found in patients suffering from diseases of the stomach, gall-bladder or pancreas. In such cases the gut wall is thinner than normal. Such a condition must be differentiated from that of megaduodenum, in which with a dilatation the muscle wall of the duodenum is hypertrophied; a condition similar to megacolon.

Cases of megaduodenum are rare; Birnbaum and Blacker[1] make no reference to it, and in the literature I have been able to find only three instances of it. One of these occurred in a child of four and a half years, and is described by Downes[2]; another occurred in a ' young adult ' and is described by Corwin[3]; and a third case is referred to by Corwin as having been seen by W. J. Mayo. The condition is stated by Corwin to be congenital in origin, probably implying that it is the result of some defect in the development of the gut wall; but Downes states that ' it is much more probable that the gut is normal at birth, and that the distension and hypertrophy result from constriction at some point, usually the duodeno-jejunal junction.'

Pathological report of case.

The body from which this specimen was obtained was that of a still-born male infant which weighed 7 lb. It had a slightly distended abdomen. On opening the abdominal cavity several loops of greatly dilated intestine presented themselves. Those loops from the upper abdomen proved to be dilated duodenum (Fig. 1), and those from the lower abdomen were dilated parts of the ileum. The duodenal dilatation was crossed by the superior mesenteric vessels, but these produced no narrowing, although the appearance of the photograph would suggest a constriction at the site of the crossing. The pyloric canal was dilated, and there was no sphincter separating pyloric stomach from duodenum. The cardiac part of stomach was normal. The duodenal-jejunal flexure was normal in its position and appearance, and there was nothing in the nature of a mesenteric band or other form of constriction such as inflammatory adhesions or enlarged mesenteric glands, as suggested by Downes, to cause the condition. The liver and gall-bladder presented no unusual appearances. The common bile duct was distended with bile and was constricted at the duodenal opening. The jejunum throughout its whole length was definitely normal in respect of size, form and structure. Three areas of ileum, however, presented dilatations similar to the dilatation of the duodenum. These dilated parts were connected together by short lengths of fibrous cord-like bands, which lay along the free edge of the mesentery, in the position usually occupied by the gut (Fig. 2). At one point in the fibrous cord there was a short length of normal gut. The large gut was normal in all its appearances and at the stage of development normally found in new-born infants. There was little or no meconium in the large gut, and there was

no content in any of the ileal cyst-like dilatations. As the specimen was examined within twenty hours of death it is certain that there had not been any absorption of fluid content.

The microscopic examination of one of the connecting bands showed it to consist entirely of fibrous tissue, in which there was no trace, as shown by differential staining, either of glandular or muscular tissue to suggest intestine. The fibrous character of the cord was so complete that it is difficult to say whether it represents a primary condition with a total absence of muscle and epithelium differentiation, or whether it is a secondary change with a degeneration of muscle and epithelium. The dilated portion of the ileum, on microscopic examination (Fig. 4) showed hypertrophy of the muscle walls, the longitudinal layer of muscle being more thickened than the circular. The two layers of muscle were not in close contact, as

FIG. 1.—Photograph of the dilated loops of duodenum and ileum which presented on opening the abdomen.

FIG. 2.—Photograph of the ileal dilatations with a band connecting one of them to a short portion of normal ileum.

in the normal gut, but were separated from one another by a well developed layer of fibrous connective tissue. The mucosa was poorly developed and formed a thin layer around the inner surface of the muscular tube. Peyer's lymphatic patches were arranged in thin elongated diffused areas. The microscopic characters of the duodenum (Fig. 5) showed a similar hypertrophy, again particularly of the longitudinal wall, and an attenuated layer of mucosa. The two muscle layers, as in the ileum, were separated from one another by a thick layer of connective tissue, and the submucosa consisted of a wide band of strong fibrous tissue, exceeding in thickness that of the circular muscle layer, and in it there was no indication of any œdematous changes. The blood vessels of the sub-mucosa of the dilated duodenum and ileum were greatly enlarged. Careful examination of the sections was made for the myenteric plexus but mere traces only of it could be found.

The ætiology of atresia of the gut is well known. It is due to a failure of the gut to form a lumen. Tandler[4] and others have shown that stenosis is the normal condition of the gut at the fifth week of embryonic life. The condition is brought about by the rapid proliferation of the primary epithelial cells which line the simple tube, and the cells quickly occlude the tube. Some weeks later in development the lumen of the gut reappears, and is formed by a fusion of certain vacuoles or cleft-like spaces which appear in the solid cord of cells. The persistence of the solid tube stage of the gut, with failure to differentiate and form the mucosa and muscle layers, results in the formation

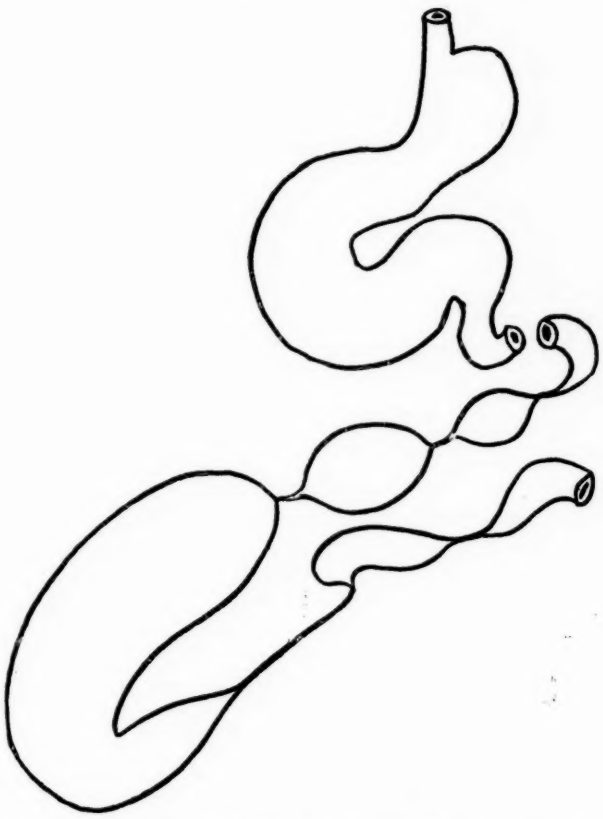

FIG. 3.—Diagram to show the general arrangement of the atresias and the dilatations of pyloric canal, duodenum, and ileum. The normal jejunum is not shown.

of bands of fibrous issue. Atresia is relatively common. Of 392 cases collected by Davis and Poynter[5] the obstruction was located 134 times in the duodenum, 60 times in the jejunum, 101 times in the ileum and cæcum, and 39 times in the colon.

It is more difficult to find an explanation for the dilated portions of the gut. These may be primary unconditioned hypertrophies, or they may be secondary conditions caused by an attempt of the gut to overcome the obstruction of the atresia. The dilatation and hypertrophy of the duodenum would suggest that the condition is a primary one, as no obstruction was present at the duodeno-jejunal junction, and the jejunum was normal from